This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

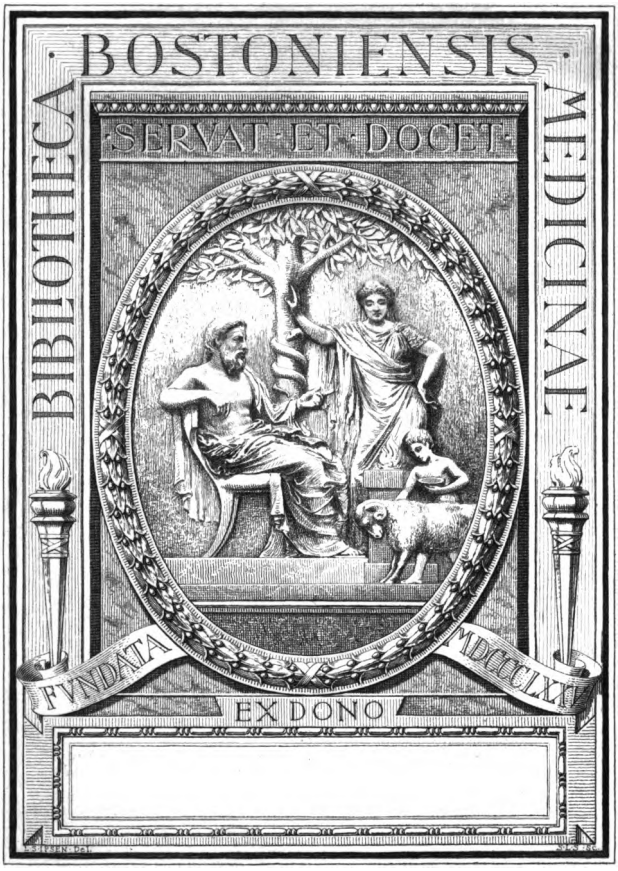
+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

COUNTWAY LIBRARY
HC 36ZH 4

Digitized by Google



Digitized by Google

Digitized by Google

Digitized by Google

THE PURIN BODIES

OF

FOOD STUFFS,

their Estimation, Action, and Significance.

BY

I. WALKER HALL, M.D.,

Assistant Lecturer and Demonstrator in Pathology, the Owens College; formerly Senior Demonstrator in Physiology at the Owens College, Victoria University.

MANCHESTER:
SHERRATT & HUGHES, 27, St. Ann Street.
1902.

Digitized by Google

J2F91

BOSTON MEDICAL
JAN 18 1904
LIBRARY.

5. No. 45

Digitized by Google

8054

INTRODUCTION.

My object in undertaking the following investigation was to obtain further information as to the action of purin bodies and their metabolism, and to discover some means whereby the early pathological changes in certain metabolic disorders may be detected.

In endeavouring to solve these problems, I have made estimations of the purin bodies present in the more common foodstuffs, and studied their specific effects upon the metabolic processes in animals and man, when they are introduced into the body subcutaneously or taken by the mouth. Were it possible to recognise the first stages of the altered functions, the application of preventive and remedial measures would soon follow. The importance of the subject is emphasised by our knowledge of the secondary results of imperfectly katabolised substances upon the vascular and excretory systems. It has been aptly remarked that two-thirds of the cases of cardiac strain in the second half of life present a history of perverted metabolism.

I desire to specially express my indebtedness to Professor Stirling, Manchester, Geheimrath Hering and Professor Siegfried, Leipzig, Professors Johansson and Holmgren, Stockholm, for permission to work in their laboratories. My thanks are also due to them, as well as to Professor Santesson, Stockholm, and Dr. Burian, Leipzig, for their kindly interest and encouragement in an enquiry, which although by no means fulfilling the projected aims, will, I hope, furnish some basis for further work in a similar direction.

Digitized by Google

Digitized by Google

CONTENTS.

CHAPTER		PAGE
1.	The Chemistry and Physiology of Food Purins	9
2.	The Methods of Estimation	16
3.	The Quantitative Estimation of Purins	28
	a. in Meats	28
	b. in Vegetables	32
	c. in Beverages	33
4.	The Actions of Food Purins	36
	a. Alimentary System	36
	b. Circulatory ,,	37
	c. Respiratory ,,	39
	d. Genito-urinary ,,	40
	e. Nervous ,,	41
	f. Skeletal ,,	41
5.	The Action of Food Purins on the Elimination of CO_2	44
6.	The Histological Effects of Continued Daily Injection of Food Purins	51
7.	The Significance of Food Purins in Metabolism	57
	a. Meats	62
	b. Vegetables	65
	c. Beer and Alcohol	68
	d. The Fæcal Purins	72
	e. The Rate of Purin Elimination	76
	f. The Exogenous Purin-remainder	77
8.	The Pathology of the Purin Bodies	81
9.	The Estimation of Urinary Purins	87
10.	Summary of Results and Conclusions	97
11.	Bibliography	100

Digitized by Google

Digitized by Google

TABLES AND ILLUSTRATIONS.

		PAGE
1.	The Earlier Estimations of Xanthins in Organs	14
2.	Later Estimations of Purins in Meats	14
3.	Comparison of the "Purin" Estimations	15
4.	Estimations of Purins in Meats	29
5.	The "Free" and "Bound" Purins of Meats	30
6.	The Total and Extractive Nitrogen of Meats	31
7.	Estimations of Purins in Vegetables	33
8.	,, ,, Beverages	34
9.	The Effect of Purins upon CO_2 Elimination	47
10.	The Absorption and Elimination of Hypoxanthin	49
11.	,, ,, ,, of Uric Acid	49
12.	Blood Pressures, after Injection of Purin Bodies	53
13.	The Endogenous Factor	63
14.	The Metabolism of Foodstuff Purins	64
15.	,, ,, Vegetable ,, W.H.	66
16.	,, ,, ,, ,, N.	66
17.	,, ,, ,, ,, M.	67
18.	,, ,, Beverage ,,	71
19.	The Purins of the Fæces	75
20.	The Maintenance of Nitrogenous Equilibrium	76
21.	The Endogenous Purin in Relation to Body-weight	80
22.	The Estimation of Urinary Purins by the Purinometer	91
22.	The Purinometer	95

Digitized by Google

Digitized by Google

BOSTON MEDICAL
JAN. 28 1904
LIBRARY.

CHAPTER I

The Chemistry and Physiology of Food Purins.

Since Kossel established the close chemical connection between nucleins and the xanthin bodies, many experiments have been made in order to determine the precise changes which occur during the metabolism of these substances in the animal organism. Few investigations, however, have been more pertinent and conclusive than the systematic enquiries which have been made into the sources of uric acid, and which have ultimately differentiated the several factors in its formation. Of these factors the group named "endogenous," still compels the acknowledgement of its unknown origin, but the other, the exogenous, appears to be directly dependent upon the nucleins

ERRATA.

Page 10. line 23.—oxalic should be oxaluric.
,, 10. ,, 23.—parabamic ,, ,, parabanic.
,, 20. ,, 27.—BaOH ,, ,, $Ba(OH)_2$

Certain portions of this work have already appeared in abbreviated form in scientific journals. As a monograph upon food-purins it was submitted to the Victoria University as a dissertation for the degree of M.D.

,, ,, ,, . ,, ,,
,, 61. ,, 12.—estimtaions ,, ,, estimations.
,, 65. ,, 6.—0·707 ,, ,, 0·1707.
,, 66. Table XV. Table N. ,, ,, Total N.
,, 67. line 10.—add . after purin-N.
,, 71. ,, 8.—1·1800 should be 0·1800.
,, 72. ,, 19.—variations ,, ,, variation.
,, 74. ,, 17.—nuclean ,, ,, nuclein.

exhaustively, it is necessary to state some of the principal facts which underlie their group reactions in order to delineate the several phases of nuclein metabolism. The purin compounds crystallise easily, are more or less soluble in the usual solvents, and can now be oxidised and reduced. *Hypoxanthin* yields small crystalline scales with sharpened extremities almost like grains of wheat. *Xanthin* may be distinguished by its thin, flat, glistening rhombic plates, *Guanin*, by small prismatic crystals or

Digitized by Google

amorphous masses, *Adenin*, by long needle-shaped prisms, and *Uric Acid* by rhombic plates. Rarer forms have been demonstrated by variations in the media and rapidity of crystallisation.

Their solubilities present the following remarkable differences:

	Hypoxanthin.	Xanthin.	Adenin.	Uric Acid.	Guanin.
WATER: Cold	1 : 300	1 : 13000	1 : 1086	1 : 16000	Insoluble.
Hot	1 : 78	1 : 1300	—	1 : 1600	„
ALKALIES:					
Weak.	Soluble	Soluble	Soluble	Soluble	Slightly Soluble.
Strong.	„	Insoluble	Insoluble	Slightly Soluble.	Insoluble.
ACIDS:	„	Soluble	„	In *certain* acids.	Soluble.

Hypoxanthin, $C_5H_4N_4O$, Xanthin, $C_5H_4N_4O_2$, and Uric Acid, $C_5H_4N_4O_3$, differ mainly in the amount of oxygen contained, and so permit certain interchanges. By reduction of uric acid with chloroform in a sodium solution, *xanthin* first appears, and is followed by *hypoxanthin;* the latter finally splits up into CO_2, NH_3 and cyanamid bodies[144]. By oxidation with ozone, cupric oxide, and potassium permanganate in neutral or alkaline solution, *uric acid* yields allantoin $C_4H_6N_4O_3$ and CO_2. On the addition of nitric acid, or potassium chlorate and hydrochloric acid, uric acid breaks up into alloxan, $C_3H_2N_2O_4$ and urea, and by further oxidation, urea and oxalic acid are obtained, with parabamic acid, $C_3H_2N_2O_3$, and oxalic acid, $C_3H_4N_2O_4$, as intermediate products. In ammoniacal copper solutions in the presence of air, uric acid yields oxalic acid and urea[128], and when mammalian blood is added to a solution of uric acid, and allowed to stand for several days at 20—25° C., the uric acid entirely disappears, and urea, oxalic acid, NH_3 and CO_2 are found to be present[61].

When nitric acid is added to guanin, $C_5H_5N_5O$, xanthin is formed; adenin, $C_5H_5N_5$, plus nitric acid, yields hypoxanthin,[84] and by pancreatic digestion, hypoxanthin, xanthin and guanin are broken up into simpler bodies, but hypoxanthin is the least altered.[8 131]

Digitized by Google

As regards their cleavage products:—

Uric Acid + HCl heated to 160°—170° C = glycocoll, NH_3 and CO_2.
Adenin + HCl heated to 180° C = glycocoll, NH_3 and CO_2 and formic acid.
Xanthin + HCl heated to 200° C = „ „ „ „
Guanin + HCl warmed = „ „ „ „

Through their affinity for silver and copper salts, the purin bodies may be fully precipitated by silver nitrate in ammoniacal solutions, or by cuprous oxide in the presence of sodium bisulphite. The separation of the several purins, although somewhat tedious, presents no special difficulties.

Amongst foodstuffs the purin bodies are widely distributed. They exist in all forms of meat extracts, in the flesh meats of ordinary consumption, and in larger quantities in the glandular organs—thymus, pancreas, etc. In lesser amount they occur in many vegetables, as oats, potato, and sugar beet. Schultze and Bossard[132] found them also present in the leaves and bark of certain trees, in young grass, and oat straw; in other plants they found a body *vernin*[133] which, on the addition of HCl, yielded a guanin. Bethe[11] states that the scales of *Alburnus lucidus* consist largely of guanin. Griffiths found that the pigment of *Dienychylus viridescens* yielded uric acid when treated with HCl, and Hopkins observed that uric acid formed the basis of the white pigment of *Pierida brassica*. That the purins were first discovered in urinary calculi, and occur as regular constituents of human urine and fæces, are facts, which although well-known, may yet be cited, because they to a great extent express the chemical activity of individual metabolism, and the normal or abnormal conditions of the organic functions.

Before the connection between the purins and nucleins was known, the former were the cause of much controversy. Lehmann,[85] Proust and many others investigated their effect upon the heart and circulation. Thudicum, in England, issued a treatise on the "origin nature and uses of Liebig's extract," in 1869, and the subject became one of distinctly polemic interest for both French and German scientists. Their conclusions attached little harmful influence to these bodies, but Gaucher,[43]

Digitized by Google

in 1886, obtained degeneration of the convoluted tubule cells of the kidney after the injection of hypoxanthin, and von Noorden,[107] in 1896, remarked upon its constant presence in the usual articles of diet, and the harmful influence its deficient excretion might exert upon the kidneys and general tissues. He considered it a matter for regret, however, that no data existed as to the purin contents of the various meats of common consumption, for quantitative determinations would have been of value in regard to the much-discussed question of "white or dark meats"—veal, chicken: beef, mutton—in gout and kidney diseases, during general convalescence, and in disorders of the stomach and intestinal mucous membranes.

Senator[134] and von Leyden[88] believe that the dark meats contain a larger amount of extractives than the lighter varieties, and hence are more irritating to the kidneys during excretion. Saundly,[125] West,[153] and Yeo[159] condemn soups and meat extracts on account of their "extractives." Dyce Duckworth[33] only allows the use of red meats once a day in nephritis; von Noorden[107] permits their use in similar quantities to the white sorts, and Yeo prefers white meats alone; Dickinson[32] advises light animal broths in nephritis, Luff[91] is disinclined to their use in acute gouty attacks, but recommends either beef, mutton, chicken, turkey, pheasant or calf's *sweetbread* once a day during the intervals.

In order to discover some tangible reasons for these opinions, Offer and Rosenqvist,[108] in 1899 analysed certain meat foods, and were led to the conclusion that the variations in the amount of extractives contained in white and red meats were so slight that any theory as to their degree of irritative action, based upon such differences could not be well founded. Their methods were not, however, free from objections, the varieties of foods they examined were but few, and even if their results were correct, it is obvious that the investigation calls for much more consideration than the simple analytical differences of the ingesta.

Digitized by Google

A deeper interest, however, centres in the specific actions of the food purins upon the various systems of the body, and their behaviour during the processes of metabolism. For such aspects are important in their relation to the construction of children's dietaries in connection with the evolution of the fermentative and chemical functions of their metabolic organs, and the tissue changes in certain diatheses. Not less significant are the action of purins in regard to the cessation of the processes of growth, the maintenance of adult life, and the gradual decline of bodily activities. Further, the *individuality* of "body" (Körper) chemistry is another factor which investigations upon these bodies has emphasised, and hence there is a need for some methods by which individual patients may be more accessible for the precise determination of their purin or nuclein metabolism.

The existent analyses of the purin-holding contents of food are best given in tabular form. They are but few in number. Hutchinson,[62] in 1900, writes: "As regards the amount of extractives, but few data are available," and their absence from scientific literature has been frequently deplored. Jerome,[65] in his admirable paper upon the relations between variations in the food and uric acid excretions, states that the alloxur-nitrogen of the food was not determined, and in the absence of precise knowledge as to the quantity of alloxur-nitrogen in both the food and fæces, certain deductions were impossible. Naturally, such estimations, together with those of the urinary purin, make too much demand upon the energy and time of one worker, but had any reliable food purin analyses been then available, from Jerome we should probably have learned of the "endogenous" and "exogenous" factors of uric acid formation, instead of noting their appearance several years later in a language other than our own.

Digitized by Google

Table I.

The Earlier Estimations of Xanthins in Food.

	Hypoxanthin %	Xanthin %	Calculated as Purin Nitrogen %	
Beef	0·022		0·008	Strecker [142]
	0·027		0·018	Neubauer [105]
	0·016		0.006	
	0·156		0·062	Stadeler [139]
Chicken	0·073		0·029	Kossel [75]
	0·129		0·051	
Fowl		0·070	0·028	Hoffmann
			0·026	
Dove	0·037		0·014	Demant [30]
	0·090		0·036	
	0·107		0·048	
	0·120		0·060	Kossel [75]
Liver—ox	0·011		0·048	Stadeler [139]
„ dog	0·082		0·033	Kossel [75]
„ ox	0·053		0·021	

Table II.

Later Estimations of "Purins" in Food.

	% of purin nitrogen.	Calculated as purin %.	
Liver—dog	0·175	0·4375	Kossel[75].
„ calf	0·123	0·3575	Burian und Schur[20].
Beef	0·053	0·1225	
„	0·030	0·0700	Kossel.
„	0·046	0·1150	Offer und Rosenqvist[108].
„ steak	0·071	0·1775	„ „ „
Veal	0·057	0·1325	Burian und Schur.
„	0·030	0·0750	Offer und Rosenqvist.
Pork	0·031	0·0775	„ „ „
Mutton	0·034	0·0850	„ „ „
Ham	0·063	0·1575	Burian und Schur.
„	0·052	0·1300	Offer und Rosenqvist.
Fowl	0·030	0·0750	„ „ „

Digitized by Google

Table III.

Comparison of the Later Estimations.

	Burian und Schur.		Offer und Rosenqvist.	
	Purin N %	Purin %	Purin N %	Purin %
Beef	0·053	0·1325	0·046	0·1150
Veal	0·057	0·1425	0·030	0·0750
Ham	0·063	0·1575	0·052	0·1300

Variations in the methods of extraction and estimation perhaps account for these marked differences. The quantities of purins found have gradually increased from the earlier to the later investigators, but even were it possible to strike an average between the widely different figures of the later results, the analyses only include a few of the substances used as national food. Any estimations of such constituents should, however, be first approached from the standpoint whence the present results have diverged, so that a critical study of the methods employed becomes a necessary preliminary to the further consideration of the substances themselves.

Digitized by Google

CHAPTER II.

The Methods of Extraction and Estimation.

As the "purin" may exist in weak combination with certain proteid substances, or their precipitation be hindered by the presence of albuminous bodies, one object of the methods employed has been to remove all proteids from the solution containing the purins before attempting to obtain the latter as metallic compounds. The following papers contain the principal processes and modifications that have been used:—

Strecker, "Liebig's Annalen," 108, 1858.
Neubauer, "Zeit. für Analy. Chemie.," 16, 1867.
Salkowski, "Virchows Archiv.," Bd. 50, S. 174, 1870.
Salomon, "Zeit. für Phys. Chemei.," Bd. 2, S. 65, 1879.
Demant, "Zeit. für Phys. Chemie.," Bd. 3, S. 387, 1879.
Kossel, "Zeit. für Phys. Chemie.," Bd. 7, 8, 1883.
Stadthagen, "Virchows Archiv.," 109, S. 399, 1887.
Burian und Schur, "Zeit. für Phys. Chemie.," Bd. 23, S. 60, 1898.
Offer und Rosenqvist," "Berlin. klin. Woch.," S. 938, 1899.
Burian und Schur, "Pflüger's Archiv.," 80, S. 24, 1900.

The earlier of these workers extracted the minced-up substance with water at 50° C. for several hours, removed the proteids with lead acetate, and then precipitated the xanthins by silver salts, and crystallised them from strong nitric acid solutions. Kossel[75] observed that extraction with a dilute solution of sulphuric acid accelerated the process and increased the yield, and so he boiled the organs in weak H_2SO_4 for some hours, then neutralised, removed the sulphate by baryta, the baryta by CO_2, and precipitated the purins by ammoniacal solution of silver nitrate. When, however, albumin is boiled for a long time in acid solution, small quantities of albumose are formed, and these enter into combination with the purin bodies, or in some way hinder their precipitation. Thus it became necessary to remove these from the solution. Kossel

Digitized by Google

employed lead acetate for this purpose; Stadthagen used a double volume of 95 per cent. alcohol, as he found that with the lead acetate method $^1/_{26}$th of the total purin was lost. Burian and Schur, after removal of the albumins, precipitated the "purins" by ammoniacal silver nitrate solution in the presence of albumoses; from the filtrate they removed the silver by SH_2, dispelled the latter by heat, and precipitated the albumoses by a mixture of equal parts of basic and lead acetate, then re-precipitated with silver and added the amount of the resultant purin to that of the former. The length of this process and the rôle of the albumoses in preventing complete precipitation, provided the cause for my endeavours to overcome these difficulties.

In selecting precipitants, it was necessary to bear in mind the ultimate introduction of silver salts. Devoto's[31] method for the separation of proteids by a saturated solution of *ammonium sulphate* seemed worth a trial. The albumoses were not, however, entirely removed, and I found that the presence of $(NH_4)_2SO_4$ hindered the purin precipitation. For both these reasons, the method was inapplicable.

Tannic acid. It was not easy to obtain this reagent free from nitrogen, so control estimations were always necessary. To entirely remove it from the solutions before using the ammoniacal silver was also a matter of considerable difficulty. The precipitates produced neither fell nor filtered easily, until the solution had stood for some days. An endeavour to hasten the process by the centrifuge was only partially successful. Although the remaining albumins were efficiently removed, the albumoses and gelatines were still suspended as flocculent flakes. An extract from boiled codfish, to which tannic acid was added on November 6th, 1900, after filtration, gave the following results by Kjeldahl's process.

(1) After boiling in weak acetic acid solution and filtering, 0,0980 % N., estimated November 6th, 1900.

(2) After boiling in weak acetic acid solution and filtering, 0,0910 % N., estimated November 6th, 1900.

Digitized by Google

(3) After adding tannic acid, 0,0908 %, estimated November 26th, 1900.

(4) After adding tannic acid, 0,0336 %, estimated June 20th, 1901.

Almén's solution[101] of 4 gm. tannic acid, 8 cc., 25 per cent. acetic acid, 190 cc 50 per cent. alcohol was tried, but as the precipitates were soluble in excess of the reagent, it was not entirely satisfactory.

Stadthagen used two volumes of 95 per cent. alcohol, but even if this process were free from objections, the high government duty upon this scientific necessity renders the process too expensive for continuous use in England.

Zinc sulphate, according to Baumann and Bönner[10] and Zuntz,[161] precipitates albumoses as perfectly as saturated ammonium sulphate solution. They add 1 cc. of 25 per cent. H_2SO_4 to each 50 cc. of the albumose solution, and then saturate it with zinc sulphate. The precipitate contains NH_3, tyrosin, small amounts of kreatin and sometimes traces of leucin, in addition to that of the albumoses, but the flesh bases are said to be untouched. The method does not admit of easy application to the estimation of purins, as the presence of zinc certainly hinders their precipitation, and its hydroxide is not too easy to dissolve. An extract of raw codfish, which gave a good biuret reaction for albumose,

By ordinary methods yielded	0·2864	% purin N.
After $ZnSO_4$ precipitation yielded	0·1081	„
An extract of rabbits' muscles		
By ordinary methods yielded	0·1536	„
After $ZnSO_4$ ppt., removal of the Zinc by SH_2 and overplus of NH_4OH yielded	0·1523	„

The removal of the zinc by SH_2 appeared to render the method somewhat more precise, but its details were difficult to modify.

Beckmann's precipitation of proteids[101] by *formaldehyde* was unfortunately quite inapplicable to the purpose in view.

Digitized by Google

Krüger and Wulff's copper process precipitates other bodies than the purins, but yet, as many observers have shown, does not always completely precipitate the xanthins. Hence, although Malfatti[95] found it reliable, it was not considered a safe method to employ. In the earlier days of the enquiry it was used as a "control," but its variable results led to its discontinuance.

As gelatinous substances are present in extracts of cooked meats, it was necessary to provide for their removal. Salkowski[101] found that flesh treated with water at a temperature not exceeding 30° C., yields no gelatine; but to obtain complete cleavage of the nucleins the minced meat must be boiled for some hours. The alcoholic method for the precipitation of gelatine is not now considered reliable, so that on the few occasions when a cold extract of meat was desired, the bromine process of Allen and Searle was employed.[3]

The results of my experiments gave so little satisfaction, that I decided to proceed upon the lines of Burian and Schur's later method, modifying it as occasion arose. When I had used it for several months His and Hagen[58] published a critical summary of the methods available for the estimation of purin bodies in animal organs. Their results led them to conclude that in the "direct and correctur" method of Burian and Schur, the first precipitate contained albumose as well as silver-purin, and hence gave a too high nitrogen result: that the albumose could be removed by zinc sulphate or ammonium sulphate, but that "purins" were carried down with the albumose precipitate, and lessened the total amount of purin-nitrogen. This confirmed my own previous results. They also considered that no reliable method for the estimation of food purins existed, for the lead acetate method gave a loss, and although this was nearly constant in the case of beef, with rabbit flesh and other meats containing methylxanthins, the yield of nuclein substances was inconstant. It became imperative, therefore, to re-examine the method I had selected. Elsewhere I have stated the detailed results of such

Digitized by Google

investigation made in conjunction with Dr. Burian ("Redaction Zeit. f. Phys. Chemie.," Mar., 1902) of which the following is a brief summary. From a critical standpoint it appeared necessary to answer the following questions:—

(1) Does the precipitate contain any nitrogenous bodies other than purins, and what is its precise chemical constitution?

(2) What conditions affect the total purin precipitation?

(3) What further modifications should be made? To fully elucidate these points, each step of the process was examined separately. (1) *Influence of the quantity of the solution and the length of the boiling.* The experimental results showed that the dilution of the solution, and the duration of boiling (above 12 hours) do not materially alter the total amount of purin obtained, but that in dilute solutions the first precipitate is small, and the second or "correctur" precipitate is larger, and if the solutions are evaporated down to 200—300 cc. before the ammoniacal silver nitrate is added, the "correctur" precipitate is so small that it may be almost neglected.

The addition of barium hydrate solution. The figures obtained from estimations of glandular and muscular tissues show that when the original solution is made weakly alkaline by barium hydrate, more albumose is present after filtration than when it is made strongly alkaline; hence after weak barium alkalinity there is a greater hindrance to the purin precipitation, and the "correctur" or second precipitate contains the greater proportion of the purin. Additionally, with a marked surplus of $Ba(OH)_2$ the introduction of CO_2 produces its more complete removal.

The evaporation of the solution before silver precipitation. Comparative estimations of the purin-contents of solutions evaporated in neutral and acid media, show that unless marked acidity is maintained, upwards of 50 per cent. of the total purins are decomposed and lost. When the solutions were very concentrated, it was found that some of the less soluble xanthins formed small insoluble masses, whose solution necessitated the addition of sodium hydrate. As this reagent

Digitized by Google

generally contains CO_2, it fulfilled an additional rôle in the removal of any remaining barium compounds.

Influence of "washing" upon the silver-purin precipitate. Very large amounts of pancreas, thymus and beef were taken, the obtained purin precipitates dried in vacuo, then decomposed by HCl and the resultant filtrate tested for the presence of albumoses. When the precipitate had been washed with *cold* water, a distinct biuret reaction was obtained, but if water at 60° C. had been employed, the biuret reaction was absent or of very faint colour. The same result appeared in quantitative estimations. If the precipitate from a peptone solution containing a known quantity of guanin was washed with cold water the purin nitrogen was always too high, but after hot washing the figures approximated the nitrogen of the added guanin and were entirely satisfactory:—

Peptone Solution. 6%.	Guanin added. Nitrogen.	Guanin N recovered. Hot washing.	Cold washing.
20 cc. in 200 cc.	0·0243	0·0228	0·0232
20 cc. in 340 cc.	0·0644	0·0604	0·0663
20 cc. in 640 cc.	0·0545	0·0561	0·0617

The examination of the precipitate itself. The washed precipitate was subjected to elementary analysis. The relation of C – N in hypoxanthin and xanthin is as 15:14, and in guanin and adenin as 12 – 14.

Estimations of sheep's pancreas.

Found		Calculated	
C	= 19·33 %.	C	= 15·66 %.
H	= 1·56 %.	H	= 1·30 %.
N	= 22·67 %.	N	= 18·27 %.
Ag	= 49·46 %.	Ag	= 56·40 %.

With the presence of ammonia and the repeated washing, the possibility exists that a small amount of silver oxide may be formed, but the proportions of C – N in guanin are 12 – 14, and in this case are as 11·91 : 14.

Digitized by Google

With *thymus*, C = 24·55.
N = 17·50 ∴ C − N = 19·6 : 14.
instead of C − N = 15 : 14.

Another estimation of *thymus*, C, 20·15.
N, 11·55 ∴ C − N = 24·4 : 14.
instead of C − N = 15 : 14.

In *muscle*, C = 23·10.
N = 14·23, hence C − N = 22·9 : 14, instead of 15 : 14.

The precipitate from pancreas thus appeared to be quite pure, but those obtained from thymus and muscle yielded too high a percentage of C, although the amount of N was correct. As in these estimations the nitrogen percentage is all that is necessary, the precipitates are therefore sufficiently pure for practical purposes. In order, however, to remove the surplus carbon, extracts of horseflesh were precipitated by phosphotungstic acid. After filtration, the solution contained a small amount of unprecipitated purins. The excess phosphotungstic acid and the H_2SO_4 were removed by $Ba(OH)_2$, and the barium precipitated by CO_2. The solution, after being entirely freed from any remaining traces of barium by the addition of ammonium carbonate, was evaporated and dried at 100° C. until constant, then weighed and decomposed. It yielded the following results: —

C = 19·35 %.
H = 1·40 %.
N = 18·12 %.
C : N = 15·5 : 14.

As the calculated proportions of C : N in xanthin and hypoxanthin are 15 : 14, the precipitate was entirely pure, and thus allowed accurate results to be obtained.

Quantitative results. The figures quoted in regard to the "washing" of the precipitate show that the guanin added to a peptone solution can be almost completely recovered by this process. The following results additionally confirm the conclusion that the method is thoroughly reliable.

Digitized by Google

	Found Purin.	Added Guanin and Xanthin N.	Total.	Found by this method.	Percentage difference.
Pancreas	0·0526	0·0362	0·0888	0·0877	– 1·3 %.
Thymus	0·1568	0·0726	0·2294	0·2287	– 0·3 %.
Beef	0·0620	0·0777	0·1397	0·1421	+ 1·7 %.
Veal	0·0713	0·0259	0·0972	0·0970	– 0·2 %.
Horseflesh	0·0551	0·0261	0·0812	0·0821	+ 1·1 %.

The modifications which appeared to hasten and facilitate the process are, perhaps, best included in a detailed description of the method. 100—150 gms. of well-hashed meat were boiled in two litres of 0·5 per cent. H_2SO_4 solution for twelve hours. The collected residue therefrom was well washed with acidulated and distilled water, and saturated hydrate of barium added to the entire filtrate until it reacted strongly alkaline. The barium precipitation removed albuminates and some albumoses, as well as the H_2SO_4. After filtration, a stream of CO_2 was led through until the fluid became weakly acid, and the total quantity was reduced by evaporation, first upon the open flame, and then on a water-bath, to about 300 cc. During this process, it was found essential to maintain marked acidity. Acetic acid was generally employed, but dilute sulphuric acid yielded similar results. A few drops of sodium hydrate were added, in order to dissolve any purins which had fallen out of solution the small amount of CO_2 present in the NaOH solution, also served the purpose of removing unprecipitated barium compounds. When the fluid had been filtered, and the residue washed successively with acidulated and distilled water, strong ammonia (0·91) was added to the filtrate, and any resultant precipitate removed. Silver nitrate solution was then used to obtain the silver-purin compounds, and after 12—24 hours, these were filtered off, well washed with water at 60° C. until the washings were quite neutral, then heated with magnesia (after Arnstein[6]), and the nitrogen contents estimated by Kjeldahl's method. The amount obtained represented the *first* or *chief precipitate*.

The filtrate from the purin precipitate was then acidified

Digitized by Google

with glacial acetic acid, the silver chloride decomposed by SH_2, the SH_2 driven off by heat, and the albumoses precipitated by a mixture of equal parts of basic and lead acetate. The precipitant was added until the solution became weakly alkaline, the removal of the albumoses hastened by the addition of a small quantity of talc, allowed to stand for 12 hours and then filtered. The excess lead was removed by dilute H_2SO_4, but unless the fluid had been previously evaporated to 300 cc., this process became very tedious. When the fluid gave neither lead nor albumose reactions, ammoniacal silver nitrate solution was added, the precipitate well washed with water at 60° C., and its nitrogen estimated by Kjeldahl's process. The amount of N. obtained represented the "correctur" precipitate, and the "chief" and "correctur" results when added together gave the total "purin."

The old lead-acetate method was used as "control" on several occasions, but always showed a slight loss as compared with the direct method. At this point the process adopted by Offer and Rosenqvist[108] may be discussed. They took 25–40 gms. of hashed flesh, added 250 cc. water, and allowed it to stand upon ice for 12–20 hours. The proteids were removed by boiling during three periods of 15 minutes each, when albumin tests were said to be entirely negative. It was deemed unnecessary to acidulate, as the flesh itself was slightly acid. After the filtrate had been made up to 500 cc., 100 cc. (5–8 gms.) were taken for the estimation of the xanthin bodies. The method was simple and expeditious, but could scarcely yield more than approximate results. The ice extracts would certainly contain the free "purins," but the bound "purins," which consist of 15 to 25 per cent. of the total amount, would be unextracted.

Further, in regard to the removal of the albuminous bodies, if the reaction of the filtrate was sufficiently acid to produce complete coagulation, it would probably, during the 45 minutes at 100° C., cause the formation of small quantities of albuminates or albumoses. On the other hand, Fürth[41] has shown that

Digitized by Google

plasma obtained from the muscles of fishes contains a proteid, myoproteid, which is not coagulated by heat, and is only precipitated by strong solutions of acetic acid; this by Offer and Rosenqvist's method would remain in solution, swell the total extractive N, and prevent the complete precipitation of the purins. There is no mention of filtration after the addition of NH_4OH, so that while the purin-N would be too small from the imperfect extraction, the presence of other nitrogenous bodies would incorrectly increase its amount. I used the method in an estimation of chicken flesh, and obtained*.....0·0262 purin N.
This compared favourably with Offer's results...0·0300 „
The *residue* from the cold extract* was then boiled with 0·5 % H_2SO_4 for 12 hours, and precipitated with $Ba(OH)_2$, the $Ba(OH)_2$ removed by H_2SO_4, and the filtrate precipitated with $AgNO_3$ and NH_4OH. The precipitate yielded 0·0144 purin N.
The *filtrate* from the first silver precipitate was acidified, the silver removed by H_2S, the filtrate heated until the H_2S was dispelled, then precipitated with a solution of subacetate of lead. After removal of the lead, $AgNO_3$ and NH_4OH were added, and the precipitate obtained yielded .....0·0087 purin N.

Thus the original result of 0·0262 N. by Offer's method
plus ... ... 0·0087 N. from „ filtrate
plus ... ... 0·0144 N. „ „ residue

gives a total of... ... 0·0493 purin N.

But estimations by the method I have employed = 0·0501 purin N.
0·0532 „ „
so that there was a loss of 0·023 N. or 0·055 xanthin bodies by Offer and Rosenqvist's method (0·0493--0·0262).

For purposes of comparison, the total nitrogen and the total "extractive" nitrogen were also estimated; 1–2 gr. were taken for the former, and 25–50 gms. for the latter. A "cold" extract was made, and the N. taken after the removal of the albumins and albuminates. The result was naturally only approximate, as the bound nucleins were unextracted, and a

Digitized by Google

small amount of albumose may have been present. The precise total extractive quantity was not, however, pertinent to the enquiry.

In relation to the effect of food purins upon the urinary excretion, it was necessary to examine the *total urinary purins.* For their amount, Arnstein's modification of Camerer's method was used.[6]

Uric acid was estimated by Hopkin's ammonium chloride process, and the *xanthins* calculated by difference. In the former case the crystals were weighed, and control studies were made by the Ludwig-Salkowski process. *Phosphoric acid* was titrated against uranium nitrate and expressed in terms of P_2O_5.

For the extraction and estimation of purins in vegetables and beverages, 250 gms.—2 kilos were boiled for twelve hours in 0·5 % H_2SO_4 solution. This was then neutralised, and the filtrate therefrom was acidified with acetic acid and again filtered. The extract was next boiled for 5—10 minutes and any resultant precipitate removed. Excess of copper sulphate and sodium bisulphite were then added, and the fluid again boiled. After standing for 2—10 hours, the precipitate was filtered off, and the residue well washed with water at 60°C. I found it of advantage to allow the precipitate to dry until " cracks " appeared, and then grind it in a mortar until it was entirely pulverised. This facilitated the next step of suspension in boiling water, and the subsequent precipitate of the Cu as copper sulphide by SH_2 or K_2S. This precipitate was washed with SH_2 water, the filtrate freed from SH_2 by heat, and evaporated to about 300 cc. Strong ammonia solution was then added, and any precipitate thoroughly removed. When the fluid gave no proteid reactions, silver nitrate was used, and the purin-N. found by Kjeldahl's process as usual. This method gives satisfactory results, except when large quantities of proteid matter are present, as in the legumes. With these it appeared safer to take the final filtrate and treat it in the same manner as that from the meat extracts, adding the two results together. The filtrate from the copper and

Digitized by Google

sodium bisulphite solution was re-precipitated on several occasions, in order to ascertain if the purins were completely removed, but I never obtained any purin-yielding precipitates upon the second addition of silver nitrate and ammonia.

The details of Kjeldahl's process are well known. Several points are, however, worthy of remark. Generally, in order to avoid contaminations, only pure English sulphuric acid was used for the destruction of the organic matter, but for the "total nitrogen" of meat this procedure was so slow that sodium pyrophosphate, as recommended by Riviére and Bailhache,[101] was employed. Before the addition of NaOH (33 %) to the $(NH_4)_2\ SO_4$ solution, the reaction was always tested, and talc used to prevent bumping. $^1/_{10}$N., barium hydrate was taken as the alkaline titrating agent, with *alizarine* as indicator for the meats, and *neutral lacmus* for the vegetable, beverage and urinary estimations.

Digitized by Google

CHAPTER III.

Estimations.

The Quantities of Purins in Meat Foods.

In mammalian muscles a considerable quantity of fat is found, and by its presence in "streaks" within the lean portions of meat, the quality of a specific "cut" or "joint" is more or less judged. The object of the breeder is, therefore, to produce in his cattle the appearances which the public prefer during the several seasons, and he obtains these by variations in the "feed" and environment. As the percentage of fat is thus changed according to the time of the year, and may vary between 1·5—29 per cent., the quantities of the other constituents will be proportionately altered. Still, analytical results show certain limits of variation in regard to the different species, and so permit of their application to scientific and clinical purposes. Additionally, the many parts of the same animal vary both in regard to their "proteid" and "extractive" percentages, and this fact should be also remembered. With fish and fowl flesh, these matters are of less moment.

The following table is constructed from my own estimations, and is intended to show the amounts of purin bodies contained in the more common of the meats found in the English dietary. The extracts were made from flesh purchased in the ordinary way from a butcher, and with the fat and other contents intact, just as they are used in the household. This was thought preferable to the separate analyses of lean and fat portions, which would have made the results inapplicable to the necessities of daily "practice."

Digitized by Google

Table IV.

	Percentage of Purin Nitrogen.	Average % of Nitrogen.	Calculated as Purin bodies.	Undried Purins. As grams per kilo.	Undried Purins. As grains per lb.
Fish:					
Cod	0·0219 0·0247	0·0233	0·0582	0·582	4·074
Plaice	0·0334 0·0302	0·0318	0·0795	0·795	5·565
Halibut ...	0·0405 0·0412	0·0408	0·1020	1·020	7·140
Salmon ...	0·0482 0·0450	0·0466	0·1165	1·165	8·155
Meats:					
Tripe	0·0235 0·0224	0·0229	0·0572	0·572	4·007
Mutton—					
Australian English...	0·0365 0·0411	0·0386	0·0965	0·965	6.755
Veal—					
Loin ...	0·0454 0·0481	0·0465	0·1162	1·162	8·137
Neck ...	0·0300				
Pork—					
Loin ...	0·0485	0·0485	0·1212	1·121	8·487
Neck ...	0·0257	0·0227	0·0567	0·567	3·969
„ ...	0·0198				
Ham	0·0505	0·0462	0·1155	1·155	8·085
* (Fat) ...	0·0419				
Beef—					
Ribs ...	0·0455	0·0455	0·1137	1·137	7·959
Sirloin ...	0·0506 0·0538	0·0522	0·1305	1·305	9·135
Steak ...	0·0826	0·0826	0·2065	2.066	14·455
Liver ...	0·1125 0·1078	0·1101	0·2752	2·752	19·264
Sweetbread—					
Thymus...	0.4025	0·4025	1·0063	10·063	70·431
Chicken ...	0·0495 0·0546 0·0512	0·0518	0·1295	1·295	9·065
Turkey ...	0·0504	0·0504	0·1260	1·260	8·820
Rabbit ...	0·0305 0·0456	0·0380	0·0970	0·970	6·314

* Considerable difficulties were experienced with this owing to the use of "curing" substances, principally potassium nitrate.

Digitized by Google

These figures call for little discussion at the moment. Certain meats appear richer in "purins" than others, but with the exception of liver and sweetbread, and when the amount of each sort necessary to provide the requisite amount of proteid or the feeling of satisfaction, is calculated, there is not much difference between the several species. More fish is generally eaten than beefsteak, if the meal consists solely of one or the other; tripe is usually a supper luxury, and sweetbread is, as a rule, taken sparsely.

But the form in which the "purins" occur is interesting in regard to their metabolism, and further investigations were undertaken in order to determine the relations of the "free" and bound "purins" in meat foods. For this purpose an extract was made with an 0·5 per cent. H_2SO_4 solution, and kept for 24 hours upon ice, as Meischer[99] has shown that there is no cleavage of nucleins when the temperature does not exceed 2—3° C. The filtrate from this extract contained the "free" purins and was treated as usual. The residue was boiled for twelve hours in 0·5 per cent. H_2SO_4, and then precipitated by a mixture of basic and lead acetate, the lead removed by H_2SO_4 and the purins obtained as silver compounds. It is possible that a small quantity of the "bound" purin was present in the first filtrate, but it may be safely neglected. Appended to the table stating the results obtained are the figures of Burian and Schur[20] and of Katz,[68] showing the relation of the phosphorus and sulphur to the "bound" purin.

TABLE V.

THE "FREE" AND "BOUND" PURINS OF MEATS.

	"*Free*" purin Nitrogen.	"*Bound*" purin Nitrogen.	P_2O_5.	Sulphur.
Cod	0·0299	0·0106	...	
Tripe	0·0143	0·0092	...	
Chicken	0·0348	0·0147	...	
Ham	0·0398	0·0064	4·8702	2·0430
Oxflesh	0·0460	0·0070	3·8944	1·8677
Veal	0·0430	0·0100	5·0291	2·2586
Liver	0·0330	0·0790	...	
Sweetbread ...	0·0420	0·3510	...	

Digitized by Google

As possessing some interest in relation to the percentage of purins already cited, and as a contribution to existent analyses, my estimations of the total nitrogen, and the total extractive nitrogen of the meats examined are given in the following table:—

TABLE VI.

	Total N.%	Total Extractive N.%		Total N.%	Total Extractive N.%
Cod ...	4·445	0·2659	Pork ...	2·576	0·2097
	4·645	0·1949		2·324	0·2151
	4·922			2·216	0·2276
Plaice ...	3·356	0·5600		2·284	
	3·040		Ham ...	4·200	
Halibut	3·370	0·3570	*dry* ...	5·840	0·4710
	3·360		...	5·640	
——			Beefsteak	4.900	0·4800
Tripe ...	2·800	0·0980		4·390	
	2·600	0·1036	Chicken	3·560	—
Mutton	3·102	0·3908	Rabbit ...	3·245	0·2450
Veal ...	3·136	0·4690		3·382	
	2·856	0·3250	Liver ...	3·122	0·2850
				2·904	

The amount of total nitrogen multiplied by the factor 6·25 will give the proteid percentage. The "extractive" nitrogen consists of creatin, creatinin, hypoxanthin, xanthin, in some instances methylxanthins, and perhaps urea, carnin, and inosinic acid.

Considerable emphasis has been laid upon the alterations produced in the amount of extractives by cooking, and one infers from the remarks of Senator[135] that the various kinds of meat may yield altered percentages of residual purins when prepared and ready for eating. Apart, however, from the fact that both "roasting" and "boiling render meat more sapient by coating its surface with extractive matter, supplying a solution of purins, etc., known as gravy, and lessening its digestibility by the coagulation of proteids, there is only a very slight decrease in the amount of total purins.

If it were customary to eat meat food, minus the gravy produced during the process of preparation, the matter might be

Digitized by Google

of moment, but as not only the gravy obtained from the meat, but also an additional " purin " contribution from some fashionable meat extract is served, it is probable that more " purin " is taken with the cooked meat than exists in the freshly-killed muscles. Hence, although the percentages of purin nitrogen are expressed in terms of the raw undried substances, they may be satisfactorily applied to the several forms in which animal food is usually partaken.

The Purins of Vegetable Foods.

It is usually stated that while vegetables contain much water, indigestible cellulose and varying amounts of proteids, their extractive matter is very small. Consequently only a few stray estimations are recorded of the purins existent in vegetable foods. Jerome[66] noticed an increased uric acid excretion after the ingestion of large quantities of asparagus, and was led to investigate its nuclein percentage, and found 0·0854 gm. in 500 gms. of asparagus (0·0171 %). The Krüger-Wulff method was employed. Burian und Schur[20] with the object of finding a purin-free food, analysed bread, potatoes, rice and cabbage, but observed " traces " of " purin bodies." The following determinations will not only confirm the general impressions but furnish precise data in regard to the nuclein nitrogen of vegetable life. The percentages are certainly small, but if vegetable food is solely used, larger quantities are required than when meat is taken. As a rule, plants have no excrêta except gaseous bodies, so that katabolic resultants of a solid nature are retained within the organism; these excretory products are, however, always removed to such localities as ensure their withdrawal from the spheres of vital activity, and are met with in not inconsiderable quantities in the bark of trees, dead leaves, cell walls, etc.[46]

Table VII. includes some of the ordinary articles of vegetable dietaries, and demonstrates the need for recognition of their purin-holding capacity.

Digitized by Google

TABLE VII.

	Quantity used for Estimations.	Purin Nitrogen %	Calculated as Purin bodies %	Grams per kilo.	Grains per lb.
CEREALS:					
Bread—white	500 grams	no trace	--	—	—
Oatmeal ...	250 „	0·0212 0·0210	0·0530	0·530	3·4563
Rice	500 „	no trace	—	—	—
PULSES:					
Peameal ...	250 „	0·0156 0·0278 0·0247	0·0390	0·390	2·5413
Beans (Haricot)...	500 „	0·0250 0·0252 0·0250	0·0637	0·6375	4·1661
ROOTS AND TUBERS:					
Potatoes ...	1 kilo	0·0008 0·0006	0·0020	0·0200	0·1400
Onions ...	250 grams	0·0031 0·0040	0·0090	0·090	0·0630
Tapioca ...	250 „	no trace	—	—	—
GREEN VEGETABLES:					
Cabbage ...	large head	„	—	—	—
Lettuce ...	„	„	—	—	—
Cauliflower ...	„	„	—	—	—
Asparagus (cooked)	700 grams	0·0086	0·0215	0·2150	1·5050

THE PURINS CONTAINED IN BEVERAGES.

So many analyses of tea, coffee and cocoa exist, that it is unnecessary to add to them. From the following table it will be seen that although no purin bodies were observed in wines, they were found in fairly large amounts in other fermented liquors. Probably their presence is due to peculiarities in the "yeasting" process. The work of Victor Lehman [85] is interesting in this regard. He took 300 gms. of yeast and allowed it to stand for 24 hours at ordinary-room temperature. The filtered fluid yielded "traces" of hypoxanthin and 0·0179 gm. guanin. Further estimations of the yeast itself at body temperature demonstrated its tendency to the formation of hypoxanthins and guanins.

Digitized by Google

The significance of these facts will be discussed under another heading. It suffices now to remark that a large percentage of the existent records of uric acid excretion are from patients or workers whose dietary included beer and porter. Table VIII. contains the amount of purins found in the beverages examined.

TABLE VIII.

	Quantity used for estimation.	Purin N %	Purin %	Purin grams per litre.	Purin grains per pint.
BEERS:					
Lager Beer ...	1 litre	0·0050	0·0125	0·1250	1·0955
	„	0·0053			
Lager drink ...	„	0·0020	0·0050	0·0500	0·1533
	„	0·0021			
Pale Ale	„	0·0059	0·0145	0·1450	1·2708
	„	0·0056			
Porter	„	0·0060	0·0155	0·1550	1·3578
		0·0062			
WINES:					
Claret	½ litre	no trace	—	—	—
Volnay	„	„	—	—	—
Sherry	„	„	—	—	—
Port ... ... (Commendador)	„	„	—	—	—

TEA, &c.*		Methyl-purins.		Per tea-cup.	
Ceylon	—	0·0164	0·0587	0·0805	1·210
Indian	—	0·0147	0·0500	0·0700	1·050
China	—	0·0107	0·0365	0·0460	0·750
Coffee	—	0·0294	0·1000	0·1100	1·700

* Calculated results from Cohn. Zeit. f. Phys. Chemie. 20. 1. 1895.

Milk, butter, eggs and cheese should be placed under the heading of animal foods, but they are best estimated by the method which was employed for vegetables, and so may be considered as a separate class. *Eggs* contain no free purin or purin-yielding substance. *Butter* and *cheese* are derivatives from milk, and so may hold "traces" of nuclein bodies. The percentages of casein and water vary in different samples of

Digitized by Google

butter, and the same remark may be applied to cheese. When the curd is present, any xanthin present in the milk will pass into the slightly acid whey, just as the process of churning or separation assists their removal from the butter, and hence only minute traces can remain in these two substances. In milk, Petren [114] was unable to find any purin bodies, but Burian and Schur [20] by *complete* removal of all the albuminous bodies found 0·0014 gm. purin-N per litre. An estimation of my own yielded 0·0020 gm. N per litre. The quantities are very small, but are probably correct, as Barthel, of Stockholm (private communication), by use of the centrifuge has enumerated at least 100,000,000 leucocytes per kilo of ordinary cow's milk.

These four articles of diet form together our most valuable means of withholding purin substances from the body, and yet allow the provision of a diet at once digestible, easily absorbed and capable of maintaining nitrogenous equilibrium.

Digitized by Google

CHAPTER IV.

The "Action" of Food Purins.

When Liebig first introduced his "extract of meat," many investigations were undertaken to demonstrate the effect of the "extractives" upon the body, but since then they have received little attention. Current handbooks upon dietetics regard them as somewhat "necessary evils," and almost negative in character. They are considered to yield no potential energy, to exert no influence upon the circulatory or nervous systems, although they may remove the feeling of fatigue and slightly aid digestion. Their excretion throws extra work upon the metabolic and eliminative organs, and hence their passage through the organism is accompanied by a loss of energy. Rarely is any reference made to their cumulative after effects, which are possibly of deep import.

Alimentary system. The food purins are powerful sapients, and both directly and indirectly increase the salivary flow. The secretion induced is, however, more watery than digestive. The gastric juice, according to Pawlow[111] is similarly affected, but Potapow-Procaitis[112] has recently observed that although meat extractives increase the total quantity, they invoke little secretion of pepsin, and only affect the production of hydrochloric acid. He fed a dog (which had a Pawlow's fistula) upon milk, boiled egg albumin, and meat that had been saturated with water, in order to remove all soluble substances. Though Pawlow had determined that the amount of gastric secretion was proportional to the digestive intensity required by the ingesta, in Potapow's experiment little peptic secretion was induced. When, however, meat-extract or dextrin was added to the other food, a copious flow of pepsin immediately followed. Mark Schnorf,[94] however, finds that *pure* dextrin does not cause peptic secretion, and ascribes the action of the ordinary dextrin to certain accompanying *extractive* bodies. Lehman[85] had

Digitized by Google

severe diarrhœic attacks after taking large quantities of Liebig's extract, but these were probably due to the mineral constituents. Pure adenin causes, in the dog, intense inflammation of the mucous membranes of the stomach and intestines.[100]

The "free" purins are readily dissolved in the acid fluids of the stomach, and thus are quickly absorbed; the larger proportion of the "bound" purins, according to the recent work of O. Loewi[89] is absorbed unaltered by the intestinal mucous membrane, and the remainder is split up by the pancreatic juices, and its phosphorus excreted in the fæces. The question as to the relation between the xanthins of the food and of the fæces, will be discussed with the results of the metabolic experiments which have been performed.

Caffeine and theobromine do not exert any direct action upon the digestive functions, but after large doses, through overstimulation of the intestinal muscles, cramp and vasomotor disturbances may occur, followed by loss of the intestinal secretions.

Circulatory system. On the vascular system, the methylpurins exert more pronounced action. The earlier workers in this field concluded that these bodies directly stimulate the heart, increase its force, lessen the pulse-rate, but cause a rise in blood-pressure. Parisot[110] ascribed the whole action to vasotonic origin, and Vinci[158] obtained, after injections into normal animals, a constant rise of peripheral vascular pressure. Cushny and Van Naten[29] consider, however, that the most characteristic features of the action of caffeine are the acceleration of the rhythm, and a decreased strength of contraction in the auricle and later in the ventricle, accompanied or followed by a lessened degree of dilatation in diastole. After 0·2–1gm., a dog's heart became irregular, and the auricles and ventricles beat arhythmically. Larger doses increased the irregularity and caused fibrillary contractions. The acceleration results from the stimulation of the so-called excitomotor apparatus of the heart. The lessened contraction may be due in part to the acceleration, and thus be considered as a secondary effect of

Digitized by Google

the increased irritability of the vasomotor area, but it may be also caused by the *direct* action of caffeine upon the muscle of the auricles and ventricles. The whole action of caffeine upon the mammalian heart thus appears to consist in a descending stimulation which begins in the excitomotor area at the junction of the auricles and great veins, extends into the auricles and finally into the ventricles. The effects can be therefore explained by *direct* action on the cardiac muscle itself, without the necessity of appealing to any nervous apparatus.

Santesson,[124] working with a cannula in the pericardium, finds that administration of caffeine causes increase of pressure and of cardiac rhythm. From the comparative effect of digitalin and cardiac poisons he concludes that as caffeine only increases the systolic part of the contractions, it exerts a direct and tonic action on the cardiac muscle. Mitchell Bruce[17] regards tea, coffee and cocoa as extrinsic cardiac poisons.

As to the "purins" proper, Gautier[44] states that *xanthin* excites cardiac muscle, but Baldi[9] found it absolutely inactive on the frog's heart, although allantoin appeared to be slightly excitatory. Adenin, however, when subcutaneously injected, strengthens the beat of the dog's heart.[100] Hedbom[52] observed an increased tonus and rhythm of the isolated heart, following the application of a watery splenic extract. Lehman,[85] after drinking varying amounts of beef extract, found no alteration in the rapidity or force of the pulse. Bunge[19] obtained similar results, but Kemmerich[69] observed a slight rise in pulse frequency. Hutchinson[62] ascribes any cardiac effects to the hot water in which the meat extracts are usually taken, or to the effects of constant sipping.

Blood. Milroy and Malcolm[102] observed in young rabbits, killed 2—10 days after the injection of 0·2–0·5 gm. of nucleic acid, an accumulation of white cells in the pulmonary capillaries, an increase in the finely granular myelocytes in the marrow and a diminution in the coarsely granular oxyphile cells. The number of granules in the cells was also lessened and the usual oxyphile granules appeared to more readily take

Digitized by Google

the basophile stains. Adenin, guanin and cytosin obtained from thymus nuclein produced similar results. In guinea pigs, however, the effects were neither so constant nor noticeable. L. Hue, in 1898 (proceedings of the Physiological Society), observed that when the Drosera was fed upon nucleic acid the basophile chromatin segments were unaltered, but an extremely copious secretion appeared, accompanied by rapid bending of the tentacles. Except very slight protoplasmic vacuolation, nuclein itself, however, produced no cytological changes.

The recent work upon the caffeine group is, however, not without interest in relation to the action of the xanthin bodies. If the former exert a tonic action upon the cardiac muscle cells, it is not unlikely that when they become demethylated in the body, they may equally as the other purins act as slight irritants to the tissues. Additionally, the purin bodies are constantly taken with the food, they throw considerable work upon the metabolic and excretory functions, and if at any time these are sub-normal, the unexcreted purins may exert cumulative effects upon the circulatory organs and the blood.

Respiratory System. In 1859, Edward Smith[137] and Hoppe-Seyler found that tea and coffee excited the respiratory functions, and caused an increased output of CO^2, the effect lasting for over an hour. In both cases, however, the experiments were made with apparatus now considered unreliable. Smith wore a mask which covered the face, and which was connected by rubber tubes with the absorptive media. The method was trying and tiring, and as Hoppe-Seyler[60] has since pointed out, gave abnormal results. No evidence exists as to the action of the other purins either upon the CO_2 output or upon the respiratory mechanism. Levén in 1868, Giraud in 1881, and Parisot in 1890[110] observed that the administration of caffeine to animals caused acceleration of the respiratory movements, but after toxic doses the respiration ceased before the heart was arrested. In tea and coffee, however, there are other bodies which cause increased respiratory exchanges, for

Digitized by Google

Binz[14] and Archangelsky[5] find that a distillate of tea and coffee, free from caffeine and thein, augments both the output of CO_2 and the number of respirations. Heerlein[53] had earlier pointed out that the coffee distillate also affected the nervous system, but Lehman and Wilhelm,[84] in 1898, considered that the distillates were inert. Still, as all aromatic substances are tissue irritants, and increase reflex excitability, it is very probable that the contents of the distillate are responsible for the observed effects. The increase in the elimination of CO_2 found in the earlier experiments may thus, in some degree, be due to constituents of tea and coffee other than caffeine and theobromin, as well as to the methods of experiment and analysis which present the objections previously noted.

Genito-urinary system. That the methyl-xanthins are useful diuretics is a fact long since appreciated. The recent works of Anthen[4] and Gottleib and Magnus[4 5] show that the methyl-purins act as direct excitants of the renal parenchyma, and incite an increased exertion of the nitrogenous elements, especially of urea and uric acid. Ach[1] finds that in rabbits they are less effective. Uric acid appears to be without effect on the renal cells, although it is quite possible it may possess some lymphagogic properties.

After the injection into rabbits of small doses of hypoxanthin, Gaucher[43] observed inflammatory changes in the kidney parenchyma, and these were confirmed later by Kolisch.[71] Croftan produced under similar conditions interstitial as well as parenchymatous nephritis, albuminuria, intertubular nephritic endarteritis, and small-celled infiltration of the intima and adventitia. These changes were accompanied by an increased blood pressure and cardiac hypertrophy. In some of the animals distinct emaciation occurred. Parallel investigations with uric acid gave negative results. Upon these observations is reared the imposing theory of the alloxur-base causation of the chronic forms of nephritis.

The aminopurins, when obtained from thymus or pancreas

Digitized by Google

are excreted in unaltered condition, but when administered in a pure form, adenin causes in the dog an intense inflammation of the tubular cells, and uratic deposits in the lumen and interstices of the renal epithelium.[100] Interesting in this relation is the work of Steudel,[140] who considers that thymin through its close connection with the hypothetical "uracil" may be akin to pyrimidin derivatives. In the hope of obtaining purin bodies by systemic synthesis, he fed a dog with methyluracil, nitrouracil, dioxypyrimidin, etc., but the urinary purins were not increased, and no toxic symptoms were observed. When, however, he added an amido-group, as di- or tri- amino-oxy-pyrimidin, 0·1 – 0·2 gms. were lethal to rats, and in the kidney tubules there was deposited an almost insoluble salt of the unchanged base.

Nervous system. In small doses caffeine and theobromine are cerebral excitants, and after toxic quantities the medullary centres are directly affected, the motor functions of the cord accelerated and the peripheral nerves, particularly the pneumogastic, paralysed.[83] Baldi[9] supposes that their action is due to the liberation of a methyl group. This group has no precise stimulative effect, the excitation being dependent upon the way in which protoplasmic activity causes its liberation, and the particular portion of the purin nucleus to which it is attached. Xanthin and hypoxanthin do not increase the excitability of the spinal cord, but Gautier[44] obtained excitation of reflexes and tetanus after the injection of hypoxanthin in *guinea-pigs*. According to Salomon and Krüger the alloxur bodies cause migraine, and after large doses of uric acid, taken for experimental purposes, I have myself had intense headache lasting for several hours.

Muscular system. The purin nucleus itself exerts a specific action upon muscular tissue, and its effect is quite independent of any attached groups. Kobert[72] and Rossbach observed that the application of hypoxanthin to frog's muscles after severe muscular efforts, caused renewed activity, and inferred that it removed the sense of fatigue and stimulated muscular tissue.

Digitized by Google

Filehne[39] noticed muscle rigidity and tetanus in frogs after hypoxanthin, and rigidity followed by cardiac arrest after xanthin. Guanin and uric acid up to 100 mgm. were inert.

Mitscherlich et Bennett and Fithene, in 1887, observed similar results with di-oxy-xanthins; and Paschkis states that the toxity becomes more intense from the hypo- to the methyl-xanthins. Johannsen, after caffeine injection in frogs, obtained muscular rigidity and loss of excitability, and by analogy between blood and muscle plasma coagulation, Klemptner thought that caffein thus evoked the development of a coagulative ferment. Although Rossbach and Hartenack were unable to confirm these results, they have since been substantiated. Leblond[83] observed that in frogs, caffein produced a period of transitory rigidity followed by tonic and tetanic convulsions and diminished excitability, and thought that the action was first upon nervous and later upon muscular tissue. Lusini[92] finds that the minimum toxic and fatal doses decrease from the mono- to the tri-methyl xanthins, and that the resistance of the muscle against fatigue increases in an inverse direction, and Féré concludes that although muscular power is for a time increased, yet caffeine causes an earlier appearance of fatigue than that which normally occurs. Baldi[9] points out that while the methyl group is responsible for the excitation, it is the xanthin or purin nucleus alone which produces the rigidity and hyper-excitability. Finally, Schmiedeberg[130] has just published the results of an interesting investigation upon the rôle played by the oxygen and alkyl attachment to the purin nucleus, and shows that whilst these groups may alter the intensity of action, the purin nucleus still exerts its own specific action upon muscular tissue.

In all "purin" experiments two conditions must, however, be always remembered; (1) that the relative actions of the several purins may be due to their varying solubilities, which in the one case allows free circulation in the lymph spaces, and in another permits only partial absorption by the individual cells; and (2) that they may form weak salts with some of the

Digitized by Google

lymph constituents, and so obtain augmented or diminished powers.

Metabolism. The purin bodies do not appear to exert any direct influence on either carbohydrate or nitrogenous metabolism. Acting through the nervous system, caffeine is said to raise the "tonus" of the tissues, and so permit the organism to utilise its reserves. Indirectly, however, they invoke an output of metabolic energy to ensure their early removal from the body. They are not used for the production of cell nuclein.

In regard to the "forced feeding" treatment of tuberculosis and the small percentage of infective disorders amongst gouty patients, it would be of value to know if an excess of purin bodies in the bloodstream increases or diminishes the *immunity* of the individual, or if there is any alteration in bacterial cultures, when purins form one of the constituents of the media.

Digitized by Google

CHAPTER V.

The Comparative Effect of Purin Bodies upon the Production of CO_2.

As presiously cited, the earlier investigators observed an increased production of CO_2 after the ingestion of tea and coffee. These increases were obtained, however, by the use of unsuitable apparatus and unsatisfactory methods of analysis. It would thus be of interest to compare the results of modern experimental methods with those already recorded, and preferably to employ pure "caffeine" instead of an infusion of coffee. With the object of making such comparison, and also to ascertain the action of the other purins upon the carbohydrate metabolism, the following studies were deviseo.

The subject of experiment rose about 7-30 a.m., walked an English mile, and still fasting, entered the Sondén-Tigerstedt respiration chamber about 8-30 a.m., and lay absolutely still upon a mattress for 30 minutes, covered only with a light felt rug. One sample of air was taken five minutes after entry, and another thirty-five minutes later. The temperature and pulse-rate were observed at the commencement and at the close of each experiment. The analyses were made by a Sondén-Peterson's apparatus.

W. H., of Manchester. CO_2.

Nov. 18, 1901, 8-0 a.m., ½ gm. Benz. Natr. Caffeinicus.
8-30 a.m., ½ gm. „ „
8-35 a.m., Temp. 36·5° C., pulse 80.
9-5 a.m., Temp. 37·8° C., pulse 78. 14·6 gms.

Sensations of warmth in abdomen and over the whole surface of the body. Intense headache, which lasted the whole day. Muscular fibrillation in muscles of the back and thigh.

Nov. 19, 8-35 a.m., Temp. 36·5° C., pulse 80.
9-5 a.m., Temp. 36·5° C., pulse 78. 11·2 gms.

Digitized by Google

Nov. 20, 7-30 a.m., 0·25 gm. Hypoxanthin in slightly alkaline solution.
8-15 a.m., 0·25 gm. Hypoxanthin in slightly alkaline solution.
8-20 a.m., Temp. 36·5° C., pulse 76.
8-50 a.m., Temp. 36·5° C., pulse 76. 11·1 gms.

Slight fulness in head, and a feeling of stiffness over the whole body.

Nov. 26, 7-45 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8-20 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8-30 a.m., Temp. 37° C., pulse 84.
9-0 a.m., Temp. 36·5° C., pulse 84. 14·3 gms.

There was distinct headache and confused ideas. Sensation of warmth in the stomach.

Nov. 28, 7-45 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8-30 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8-33 a.m., Temp. 37° C., pulse 84.
9-3 a.m., Temp. 36·5° C., pulse 84. 11·6 gms.

Slight headache, sensory disturbances in abdomen.

Nov. 29, 7-45 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8-15 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8-26 a.m., Temp. 36·6° C., pulse 84.
8-56 a.m., Temp. 36·5° C., pulse 72. 14·3 gms.

Fulness in head; loss of muscular sense; confused ideas.

Nov. 30, 7-45 a.m., 0·5 gm. uric acid, entirely dissolved in sodium carbonate solution.
8-15 a.m,. 0·5 gm. uric acid, entirely dissolved in sodium carbonate solution.
8-35 a.m., Temp. 36·8° C., pulse 90.
9-5 a.m., Temp. 36·5°C., pulse 76. 11·0 gms.

Digitized by Google

Dr. Friherre A. C., of Finland.

Normal rest value 9·8
10·3
10·0
} gms. 10·3 gms.

Nov. 21, 7-30 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8-0 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8-5 a.m., Temp. 36·1° C., pulse 60.
8-35 a.m., Temp. 36·1° C., pulse 60 (fuller) 10·7 gms.

No headache; slight warmth over whole skin area; no fibrillations.

Nov. 25, 7-30 a.m., 1 grm. Benz. Natr. Caffeinicus.
8-0 a.m., 1 grm. Benz. Natr. Caffeinicus.
8-12 a.m., Temp. 36·2° C., pulse 62.
8-42 a.m., Temp. 36·2° C., pulse 62. 12·3 gms.

Headache; abdominal warmth, followed by diarrhœa in the later part of the day.

Professor Santesson, of Stockholm.

Dec. 5, 8-25 a.m., Temp. 36·8° C., pulse 56.
8-55 a.m., Temp. 36·8° C., pulse 56. 10·5 gms.

Dec. 7, 7-55 a.m., ½ gm Benz. Natr. Caffeinicus.
8-25 a.m., ½ gm. Benz. Natr. Caffeinicus.
8-35 a.m., Temp. 37·0° C., pulse 56.
9-5 a.m., Temp. 37·1° C., pulse 57. 12·5 gms.

Audition slightly augmented, also sensation of body warmth. No headache.

I am much indebted to Professor Santesson, of Stockholm, and Dr. Friherre Axel Cedercreutz, for these experiments. The readiness with which they underwent the necessary inconvenience and trouble, was only equalled by the interest they manifested in the investigation.

Digitized by Google

Table IX.

Respiration Experiments.

Date.	Time.	Volume of outflowing air.	In respiration chamber. Thermometer. Dry.	Damp.	Barometer. Corrected.	Temp.	CO_2 gms.	Remarks.	
Nov. 18 ...	8.38 a.m. ...	*Cbm.* ...	17·6 ...	9·0 ...	749·9 ...	17·0	—	...	
	9.5 ,, ...	5·3 ...	18·5 ...	9·7 ...	745· ...	16·8	14·6	...	1 gm. Caffein Nat. Benz. Walker Hall.
,, 19 ...	8.35 ,, ...	— ...	18·1 ...	9·4 ...	749·9 ...	15·8	—	...	
	9.5 ,, ...	5·2 ...	19·0 ...	10·1 ...	745· ...	16·0	11·2	...	Normal rest day. ,, ,,
,, 20 ...	8.20 ,, ...	— ...	19·8 ...	10·0 ...	746·7 ...	17·4	—	...	
	8.50 ,, ...	5·3 ...	20·8 ..	10·8 ...	745· ...	18·8	11·1	...	½ gm. Hypoxanthin. ,, ,,
,, 21 ...	8.5 ,, ...	— ...	18·1 ...	8·9 ...	744· ...	16·1	—	...	
	8.35 ,, ...	5·3 ...	19·1 ...	9·6 ...	740· ...	16·4	10·7	...	1 gm. Caffein. Nat. Benz. Dr. Friherre C.
,, 25 ...	8.12 ,, ...	— ...	19·3 ...	9·1 ...	763·9 ...	16·2	—	...	
	8.42 ,, ...	5·3 ...	19·5 ...	10·2 ...	760·8 ...	16·9	12.3	...	2 gm. ,, ,, ,, ,, ,,
,, 26 ...	8.30 ,, ...	— ...	19·6 ...	10·4 ...	758·7 ...	17·9	—	...	
	9.0 ,, ...	5·3 ...	19·6 ...	10·6 ...	760·3 ...	18·1	14·3	...	½ gm. Uric Acid. Walker Hall.
,, 28 ...	8.33 ,, ...	— ...	19·8 ...	10·9 ...	739·7 ...	18·0	—	...	
	9.3 ,, ...	5·1 ...	19·8 ...	11·0 ...	735·3 ...	18·8	11·6	...	½ gm. ,, ,, ,,
,, 29 ...	8.26 ,, ...	— ...	18·3 ...	8·9 ...	752·9 ...	16·9	—	...	
	8.56 ,, ...	4·9 ...	19·2 ...	9·6 ...	750· ...	17·	14·3	...	1 gm. Caffein. Nat. Benz. ,, ,,
,, 30 ...	8.35 ,, ...	— ...	19·25 ...	9·45 ...	750·7 ...	17·3	—	...	
	9.55 ,, ...	4·9 ...	19·35 ...	9·6 ...	747·7 ...	17·5	11·0	...	½ gm. Uric Acid. ,, ,,
Dec. 5 ...	8.25 ,, ...	— ...	18·9 ...	9·4 ...	763·9 ...	16·8	—	...	
	8.55 ,, ...	5·0 ...	19·05 ...	9·65 ...	760·8 ...	17·1	10·5	...	Normal rest day. Prof. S.
,, 6 ...	8.35 ,, ...	— ...	19·7 ...	11·0 ...	750·7 ...	17·0	—	...	
	9.5 ,, ...	5·2 ...	19·8 ...	11·25 ...	746·1 ...	17·0	12.5	...	1 gm. Caffein. Nat. Benz. ,,

To Väkmästare Jarl I am indebted for taking the samples of air during my experiments in the respiration chamber, and to Cand. phil. Fröken Rosenberg for analyses under similar conditions.

Digitized by Google

The results show that my early morning CO_2 excretion is about 11 grammes per half-hour. The values of Professor S. and Dr. C. are slightly lower, as also their pulse-rate and temperature. With Professor S. and myself, caffeine caused an increased production of CO_2 in average doses, but an almost toxic dose was required before any effect was manifest upon Dr. C. It would appear, therefore, that although caffeine may incite increased combustion, it acts variously upon different individuals. The results recorded do not indicate whether the excitation is directly upon the metabolic organs, or indirectly through the nervous system. At all events, caffeine increases the elimination of CO_2 in many cases—a fact of interest in the consideration of the treatments available for the alterations of cell-activities during " stasis."

Hypoxanthin, the monoxypurin, yielded negative results, and uric acid, the tri-oxypurin, may probably be placed in the same category. The clinical constitution of these bodies and their behaviour during metabolism, pointed to the probability of their inactivity, but the observations are of additional value, because they indicate the limited rôle of the purin-nucleus, and allow us to ascribe the effects of caffeine upon respiration to the action of its methyl-groups. That the latter mainly excite nervous tissues is certain, but that they may also directly affect cellular processes is not disproved and not improbable. In these experiments, however, the action of uric acid was somewhat irregular! On the first occasion, when the uric acid was only partially dissolved, there was a sensation of warmth in the alimentary canal, which, apart from the intense headache, would be sufficient to account for the rise obtained.[127] There was no headache on the second day, but the stomach sensations were present; for the third experiment the uric acid was completely dissolved in 340 cc. of Na_2CO_3 solution, and neither headache nor other symptoms interfered with quiet respiration and general comfort. Possibly also my body became rapidly tolerant of the repeated doses of uric acid.

Digitized by Google

That the substances taken were duly absorbed appears from the following records.

TABLE X.

Nov. 21, 7-30 a.m.
„ 21, 8-15 a.m. } 0·5 grm. Urine passed: Hypoxanthin (0·20 purin N.)

	Urine.	Total N.	Total purin Nitrogen.
10 a.m.—12 noon	415 cc.	... 3·949	... 0·1227
12 noon—3 p.m.	100 cc.	... 2·548	... 0·0792
3 p.m.—7-30 a.m., Nov. 22....	650 cc.	... 12·989	... 0·1560
	1165 cc.	19·486	0·3579

TABLE XI.

Nov. 27, 7-30—8-0 a.m., 0·5 gm. uric acid (0·166 purin N.)

	Urine.	Purin Nitrogen.
	10 a.m., 250 cc.	0·0330
	2 p.m. 150 cc.	0·0396
	8 p.m., 230 cc.	0·0606
	10-30 p.m., 150 cc.	0·0376
Nov. 28.	7-30 a.m., 270 cc.	0·0712
	1050 cc.	0·2420

It will be seen from later experiments, Table XIV., that my endogenous purin N is 0·1625 per 24 hours. During these experiments I ate purin free food, with the exception of 300 gms. of veal on the hypoxanthin day. This latter yields about one half its purin nitrogen as urinary purin N (Table IV.), therefore, 0·075 of the urinary purin would be from this source, 0·1625 + 0·075 = 0·2375. The total quantity of urinary purin after the hypoxanthin was 0·3579. This, minus 0·2375 = 0·1224 N, directly due to the hypoxanthin ingested. As the N of 0·5 gm. hypoxanthin = 0·200, it will be seen that 60 per cent. of the hypoxanthin N was voided in the urine during the 24 hours. This confirms similar results by Burian and Schur.[20] Equally, when the uric acid was taken, the purin

Digitized by Google

N excretion was 0·2420. On this day, only eggs, milk, bread, cheese and butter were eaten, and the endogenous purin N = 0·1625. The difference of 0·0795 came, therefore, from the uric acid: 0·5 gm. of uric acid = 0·1666 N, and hence during the succeeding 24 hours 47·7 % of the uric acid ingested was excreted as urinary purin. It will be shown later, that the remainder was probably excreted as urea.

A noticeable feature of these absorptive results, is the rapidity of their excretion. Almost the whole of the expected amount of hypoxanthin was eliminated as urinary purin within four, and the uric acid within eight hours. The administration of these substances might thus be of use as indicators of individual purin metabolism. A known quantity given with purin-free food, should, under ordinary circumstances, be excreted as urinary purin to the extent of 50 per cent. in "x" hours. The integrity of the liver and kidney cell activities would be a matter of easy induction from the results obtained, and some clue would be gained as to the tonicity of the vascular circulation in these organs. The application of this suggestion will appear more feasible when "purin" metabolism has been discussed.

BOSTON MEDICAL
JAN 18 1904
LIBRARY

Digitized by Google

CHAPTER VI.

The Effect of Continued Daily Injection of "Purin-Bodies" in Rabbits.

These experiments were undertaken with the object of recognising any changes in the liver, marrow and nerve cells after the injection of hypoxanthin, etc., and of repeating previous investigations upon the kidney and blood-pressure.

Dosage. The quantity injected corresponded with the average daily amount (per kilo) taken by an adult man in his food. This would be a large dose for an animal accustomed to small quantities of purins in its food, but yet not approach the "toxic" doses of Milroy and Malcolm.[102] It is difficult to conclude how much Croftan administered, as no weights of the animals are stated. The results of toxic injections are certainly of value and interest, but as the "purin" action under general conditions is perhaps more cumulative than momentary, and produces its effect indirectly through "overstrain" of cellular function, an attempt to obtain some less pronounced tissue changes should lead to a better knowledge of cell reactions in pathological metabolism.

The injections were made and the operations performed at the Carolinska Institute, Stockholm. Four young rabbits were procured six days before the experiment commenced, kept in separate cages, fed on the same daily quantities of oats throughout, and their urine examined regularly.

Each day from September 23rd to November 12th inclusive,

Rabbit 1 (1480 gms.) received 5cc. of 0·025 slightly alkaline solution of hypoxanthin = for a man weighing 68 kilos, 1·133 gms.

„ 2 (1546 gms.) received 1 cc. of 0·025 slightly alkaline solution of hypoxanthin = for a man weighing 68 kilos, 0·2193 gms.

„ 3 (1713 gms.) 1 cc. of 0·025 of guanin solution = for a man weighing 68 kilos, 0·2193 gms.

„ 4 (2025 gms.) was employed as a "control."

The hypoxanthin used for No. 1 was made by the usual

Digitized by Google

process from fresh beef, that for No. 2 was obtained from E. Merck., of Darmstadt. The guanin was prepared from the scales of *alburnus lucidus*, which, after boiling in 5 % NaOH for four hours, precipitation with freshly-prepared cuprous oxide, and decomposition by H_2S, yielded guanin upon the addition of strong ammonia to the H_2S freed filtrate. It contained 42·46 % nitrogen (Kjeldahl) and was free from all albuminous material.

The rabbits were weighed each day at 3 p.m. Although they all received the same amount of food, while the control animal had gained 145 grams at the end of the experiment, No. 1 and No. 3 had only increased 60 and 52 grams respectively, and No. 2 had lost 206 grams. The effects of the injections were thus reflected in their weights. The control rabbit increased gradually in weight, No. 1 gained up to a certain level and then just managed to maintain it, but No. 2 during the last 20 days rapidly emaciated. The animal in which guanin was injected was subject to marked diurnal or di-weekly increases or diminutions of weight.

On the 43rd day blood films were made from each rabbit, and on the following morning, blood pressure tracings were taken. After death, the heart, vessels, kidneys, lungs, marrow and brain were removed for histological examination, and the muscles boiled with 0·5 % H_2SO_4 to ascertain their purin contents. These did not differ from the average normal amount.

On November 13th, Professor Johannsen very kindly assisted me in the necessary operations for the record of blood pressures. To avoid any circulatory depression, only local anæsthetics for the skin incision were used.

Digitized by Google

Table XII.

Blood Pressure Results, November 13th, 1901.

Rabbit I. 1540 gms.	Rabbit II. 1340 gms.	Rabbit III. 1765 gms.
1-25 p.m., 104 m.m.	2-10 p.m., 100 m.m.	2-50 p.m., 104 m.m.
1-30 p.m., 106 m.m.	2-15 p.m., 104 m.m.	2-51 p.m., 98 m.m.
1-40 p.m., 110 m.m.	2-20 p.m., 102 m.m.	2-52 p.m., 96 m.m.
1-45 p.m., 108 m.m.	2-27 p.m., 106 m.m.	2-55 p.m., 96 m.m.
	2-30 p.m., 98 m.m.	

Rabbit IV. 2110 gms.	
12-10 p.m., 122 m.m.	
12-15 p.m., 116 m.m.	
12-40 p.m., 118 m.m.	
12-43 p.m., 118 m.m.	10 cc. hypoxanthin (0·5 %)
12-44 p.m., 122 m.m.	solution injected.
12-45 p.m., 122 m.m.	(intravenous).
12-50 p.m., 118 m.m.	
12-51 p.m., 118 m.m.	
12-52 p.m., 118 m.m.	

These figures show that the blood-pressure is not altered by purin bodies, either immediately or remotely. Croftan,[28] however, states that cardiac hypertrophy and increased blood-pressure are caused by the "alloxuric bases." In his rabbits he found blood pressures of 104, 107, and 108 m.m. My results are practically the same, but Croftan takes the normal B.P. at 60—90 m.m., and so shows an increase of 20—40 m.m. The "pressure" in my control animal was 120 m.m., but unfortunately Croftan's figures cannot be compared, as he does not record the weights of his rabbits. I have examined the reports of some 500 experiments conducted upon English, German and Scandinavian rabbits, and their average B.P. is 100—135 m.m. Croftan's figures thus appear to point an entirely opposite conclusion to the one he draws.

Digitized by Google

Post-mortem Appearances.

Rabbit I.

Heart: pale and flabby; no apparent increase in size.

Lungs: normal.

Liver: hæmostasis.

Kidneys: cortex very pale, little distinction between the two zones.

Marrow: pale and anæmic.

Rabbit II.

Heart: pale and flabby.

Lungs: normal.

Liver: hæmostasis.

Kidneys: same as No. 1.

Marrow: pale.

Rabbit III.

All the organs appeared normal, except for a slight hæmostasis at the base of the right lung.

Blood-films. The blood was obtained from the median artery of the ear, the films fixed with formalin vapour, stained for two minutes with 1 % saturated watery solution of methylene blue and differentiated with a 1 % solution of erythrosin and absolute alcohol. All the histological appearances are described in comparison with those of the control animal. In rabbits 1 and 2, the lymphocytes and polynuclear leucocytes were markedly increased, and cells which appeared of a basophile character were very numerous. There were no changes in the red blood corpuscles.

Histology. The following organs were examined: liver, lungs, heart, kidneys, marrow, blood vessels, and brain. They were fixed (1) in absolute alcohol, chloroform and acetic acid; (2) in mercuric chloride and glacial acetic acid; and (3) in mercuric chloride and picric acid. Afterwards they were passed through 30, 40, 50, 60, 70, 82 and 95 per cent. absolute alcohol and carbon bisulphide, carbon bisulphide, carbon bisulphide and paraffin, and finally embedded in 58° C paraffin. Sections of 2—5 (u) were cut, and coloured with the following stains:—

Digitized by Google

(1) Hæmatoxylin and eosin.
(2) „ „ orange G.
(3) Methylene blue (1 %) and eosin (½ % in alcohol).
(4) Iron alum hæmatoxylin, acid fuchsin and orange G.
(5) Toluidin blue (2 %) and differentiation in ½ % erythrosin.
(6) Weigert's fuchsin—resorcin solution.

In rabbit 1 and 2, the heart, large blood vessels and nerve cells did not show any distinct changes. In rabbit 2, there was a slight bronchitis, and the brain was hyperæmic.

The *kidneys* of both animals showed degenerative changes in the cells of the convoluted tubules, marked hyperæmia, a commencing proliferation of the intima of the *smallest* arterioles, but no interstitial nephritis, and no glomerular changes.

The *marrow* was crowded with lymphocytes and nucleated red-blood corpuscles. The cell forms intermediate between the lymphocyte and myelocyte were numerous, and showed a tendency to take basic dyes. Most noteworthy was the unusually large number of " Riesen-zellen," some of which showed signs of degeneration. Stained by Weight's resorcin method, the intima of the smaller blood vessels appeared to be " thickened."

Liver. Hyperæmia was well marked in both livers, but there were no signs of any interstitial changes. Stained with eosin and methylene blue, many scattered foci could be seen where the eosin had stained more deeply than normal, the result, probably, of slight degeneration.

In other places the capillaries were very dilated, the cells had almost lost their " row " arrangement, and were separated on all sides by the engorged vessels. Stained with iron-alum, hæmatoxylin, acid, fuchsin, orange method, the cells in these areas showed an altered arrangement of their protoplasm, which was gathered chiefly around the nucleus. The remaining hyaloplasm appeared to be " clumped " together as if " upshaken " by a strong electric current, and showed degenerative changes. There was considerable vacuolation. The nuclei

Digitized by Google

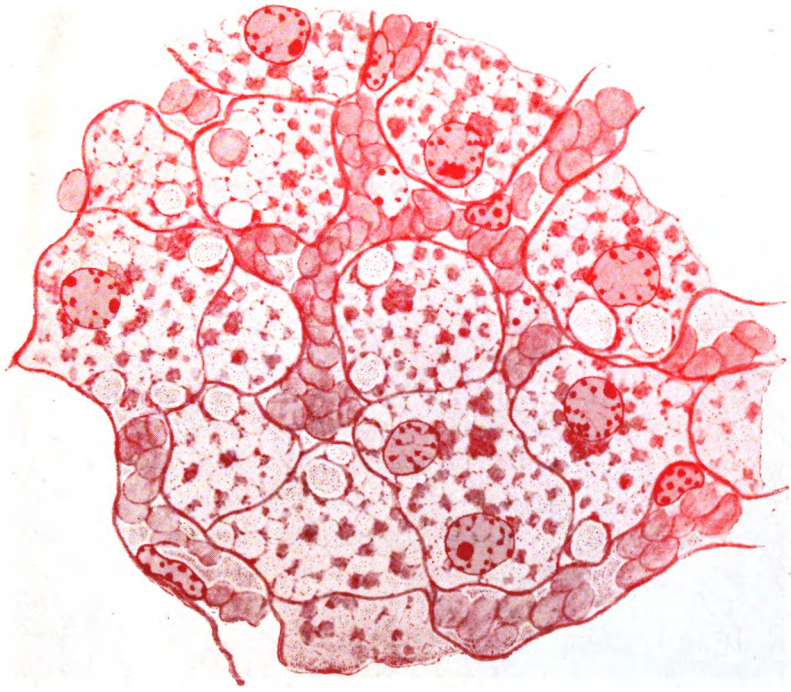
were swollen and distorted and contained sharply-staining nucleoli, principally "polar," and a large number of cells had two nuclei.

It was possible also to find odd cells which were almost necrosed, and around which leucocytes had collected. Methylene blue showed some extrusion of "nuclear" bodies. The portal canal and bile ducts were normal.

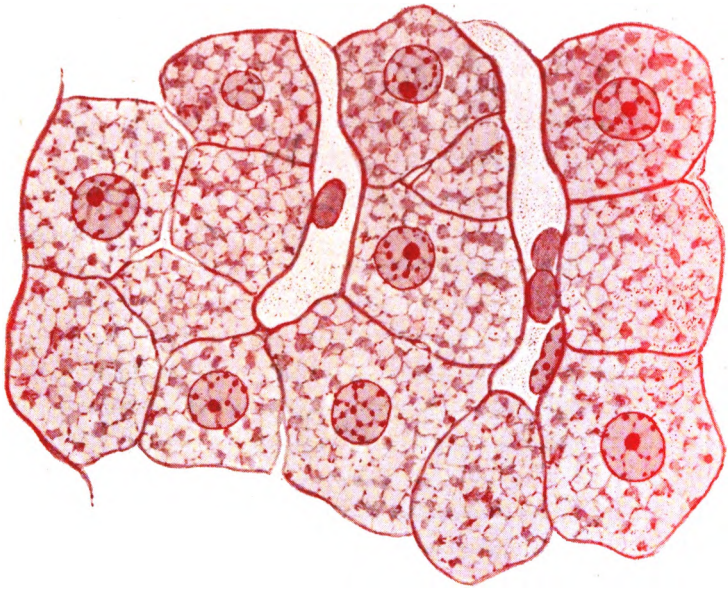
The pathological changes in the cells of the blood, lymphoid and myeloid tissues do not yet permit of full explanation, as Dominici's[35] recent experiments and criticism of Ehrlich's theories tend to show. But one may make the general inference that in these rabbits a distinct cellular reaction was directed against bodies of a toxic nature. The alterations in the liver parenchyma suggest the presence of some exogenous or endogenous cell poison, but certain morbid manifestations may follow overworked or overstrained organs, and the slowness of growth in rabbits 1 and 2 is somewhat significant.

Whether it is reasonable to assume that the purins exert a similar influence in man is hard to say. The exogenous purin in the rabbit is, as a general rule, very much less than in man; and it could be well argued that human cellular tissues are more immune to these bodies than those of the rabbit. Mitchell Bruce,[17] Bier,[13] Jelks, and many others think that the kidney, cardiac and vascular changes in nephritis are due to an irritant of a chemical nature. Croftan[28] and Kolisch consider that alloxuric bases are the specific irritant, but there is no evidence obtained by reliable methods which substantiates any increased circulation of these bodies in the blood or lymph stream of nephritic patients. At the same time it must be admitted that the purin bodies are not wholly harmless, and any deficiency of oxidation might readily delay the transformation of these insoluble substances into the soluble urate, and so retard their passage into the circulation. The tissues of rabbit No. 3, into which guanin was injected, presented no noteworthy alterations.

Digitized by Google



Liver (rabbit, after hypoxanthin injection), shewing hyperæmia—degenerative changes in the protoplasm—vacuoles—swollen and distorted nuclei—regeneration.



LIVER (RABBIT) NORMAL.

Drawn by Fröken Johansson,
Artist to Histologiska Afdelningen.
Carolinska Institutet, Stockholm.

Zeiss $\frac{1}{12}$th Immersion.
Oc 5. Camera lucida.
Iron—alum—hæmtoxylin—acid—fuchsin—orange.

Digitized by Google

Digitized by Google

CHAPTER VII.

The Significance of Food Purins.

The relation between the ingested food and the simplified nitrogenous bodies of the urine and fæces has been the cause of many experiments and much theory. Practical demonstration of the intermediary stages has been very slow, and it is only within recent years that chemical physiologists have demonstrated the differences between the decomposition products of proteid and nuclein. The view, that during metabolism one portion of the absorbed proteid is first anabolised into living protoplasm and the other undergoes direct katobolism, has been further developed by the belief that as all metabolic processes are intracellular, no katabolism occurs except as a result of cell influence. Whether, however, as some cytologists affirm, the proteid first undergoes entire anabolism, or as others consider, is in part *directly* katabolised, in both cases, as a result of cellular control, through oxidations, dehydrations, decompositions and perhaps also by synthesis, it is excreted as urea.

Wöhler and Frerichs,[154] in 1848, from the results obtained by the injection of uric acid into rabbits, considered it to be an intermediate body between proteid and urea. Amongst many others, Stadthagen, in 1887, thought that uric acid resulted from the cleavage of albumin, and that its quantity was dependent upon the amount of albumin in the food, and Mares[96] proposed a theory of its formation by glandular cells, and its consequent expression of the secretory activity of the body. As late as 1898, Luff[91] wrote: "The production of uric acid depends upon the ingestion of proteid matter, and it makes no difference whether the proteid matter be of animal or vegetable origin. The only reason that a vegetable diet is less productive of uric acid than an animal diet, is in the fact that the former is poorer in proteid material.

Digitized by Google

With the same intake of nitrogen in the two diets, there is practically no difference in the uric acid output."

On similar grounds is based Latham's theory[81] that uric acid results from the synthesis of urea and glycocin through the stages of hydantoin and biüret. In 1899, however, Taylor[146] showed that the amount of ingested proteid has absolutely no constant relation to the quantity of uric acid excreted, and that even with a proteid-free diet of 350 gms. sago, 50 gms. sugar and 100 gms. butter, the urine contained 0·273 gms. of uric acid per 24 hours, and Hess and Schmoll[56] have found no increased purin excretion after the consumption of even twenty-four hen's eggs. Jerome[66] concluded from the results of a long experiment, that "there is at present no proof that uric acid can arise in man independently of a substance containing an alloxur or purin group." Maurel,[97] however, points out that if a condition of *nitrogen hunger* is induced, the amount of nitrogen in the food exerts some influence upon the uric acid output.

Food of 3250 calories with 1·25 gm. N per day = 0·21 uric acid.
„ 3800 „ 1·00 „ „ = 0·11 „
„ 2500 „ 0·50 „ „ = 0·07 „

But as early as 1867 Gorup recognised the importance of the nuclein bodies in regard to metabolism, and Kossel,[73] in 1881, obtained xanthin bodies from pure nuclein by hydrolytic cleavage. His declaration in 1882,[84] that nuclein might be a source of uric acid with hypoxanthin as an intermediary body led to a series of experiments in which rabbits, cats and dogs were fed with nuclein bodies, and increased amounts of urinary purins demonstrated. The work of Horbaczewski,[61] who showed that oxidation of fresh splenic pulp by bacterial agencies, produced uric acid of the same N value as that of the nucleins of the tissues, and that nuclein prepared from splenic substance, dissolved in weak alkali and digested with blood at 40° C, yielded uric acid, led up to the theory that the decomposition of nuclein in the tissues yielded xanthin and hypoxanthins, and that these bodies, after oxidation, were excreted as uric acid. The in-

Digitized by Google

creased amount of uric acid, eliminated after meals rich in nucleins and present in the urines of leucocythæmic patients, supported Horbaczewski's further contention that disintegration of leucocytes was the source of uric acid.

Parallel examinations of the blood and urine have, however, subsequently shown that leucocytosis does not regularly accompany increased uric acid excretion, and Hutchinson and Macleod[63] have recently reported a case of leucopenia in which the alloxuric urinary nitrogen was of an average normal amount. Whilst we may thus regard leucocytic destruction as one source of urinary purin, it probably does not play such an important rôle as was formerly thought.

The supposition of Haig[50] that the urinary uric acid is in part the expression of the "uric acid" contained in the food, may be cited in passing. In the later editions of his book the figures which previously expressed the amount of uric acid in meat are placed under the heading of "uric acid *and xanthins.*" A few instances of the marked variations in the amount of uric acid and xanthins present, and their comparisons with the figures in Table V., necessitate the inevitable queries, how much xanthin? how much uric acid? and what method is responsible for the results?

Percentage amount of uric acid and xanthins (not nitrogen: Haig).

Lamb	0·050.	Mackerel	0·032.
Mutton	0·020.	Plaice	0·003.
Veal	0·049.	Herring	0·004.
Beef	0·016.	Rabbit	0·015
Liver	0·091.	Fowl	0·024.

The observations of Weintraud,[150] Mayer,[98] Umber,[157] Jerome,[65] and Hopkins and Hope[59] have proved that thymus and pancreas, bodies rich in nucleins, cause an increased urinary purin output.

As regards the *cleavage products of nuclein*, Strauss, in 1896,[143] obtained an increased uric acid excretion after the

Digitized by Google

addition of 50 gms. of meat extract to his usual diet, and Jerome[66] recorded varied increases after lamb, steak, mutton, fowl, pigeon and partridge.

The methyl-purins of the food increase the total purins of the urine, but only slightly affect the uric acid excretion. Albanese,[2] and Krüger and Schmidt[79] have shown that these bodies are de-methylated during metabolism, and that the 1—3—7 methyl-xanthin caffeine is excreted as di- and mono-methyl-xanthin, and the 3—7 di-methyl-xanthin, theobromin, as 3 methyl-xanthin.

All foods containing purin bodies thus appear to increase the excretion of uric acid and the xanthins. What then, is the effect of the purin-free or purin-poor foods? Milk was found by Burian and Schur[21] to result in a very low uric acid excretion, and casein, aleuronat, and sanose by Rosenfeld and Bornstein,[121] Brandenburg,[18] and Schreiber[128] to produce a similar effect. In this regard the purin excretion of children fed upon milk is interesting. Camerer[24] reports the case of an unweaned child nearly twelve months old, whose total daily urinary purin = 0·035 gm. N, and Bendix[12] obtained 0·098 gm. of uric acid (0·033 gm. N) from the urine of a three month's old child fed upon cow's milk, and in a child aged seven years I have found 0·1072 – 0·1170 urinary purin N on a milk diet, and 0·1570 – 0·1600 on a mixed diet. Camerer[24] estimated the effects of food upon purin excretion and arrived at the following results:—

	Urinary Purin N.
A diet of milk, cream, eggs, meat and coffee	= 0·72.
,, peas, cabbage, fat, bread, butter and beer	= 0·41.
,, potatoes, chesnuts, fruits, bread, butter, honey, meal and milk	= 0·36.
,, meat, fruit, vegetables, eggs and cheese	= 0·66.

As coffee and beer were taken, it is impossible to draw any adequate conclusions as to the exact effect of these diets. Taylor[146] found:—

Digitized by Google

	Uric acid.	Bases.
On a mixed normal diet	0·364	0·0249
„ „ „ plus sweetbread	0·871	0·0271
„ much proteid	0·456	0·0134
„ peas and beans	0·462	0·0195
„ nitrogen free food	0·273	0·0066

Hence we may infer that with certain foods a minimum of uric acid is excreted, and that as such foods probably contain little "purin," they make but a slight addition to the uric acid which the body produces independently of the nucleins of food-stuffs. Sivén,[136] in 1900, on a fixed diet, furnished experimental proof of such a conclusion, but to Burian and Schur is the credit due for its substantiation through their estimtaions of the purin-N of certain foods. Their results are as follows:—

	Purin nitrogen.		Purin N.
Brown bread	0·010 %	Thymus	0·386 %
Milk	0·004 %	Liver	0·123 %
Potatoes	0·0005 %	Beef	0·063 %
		Veal	0·057 %
		Ham	0·063 %

In white bread, rice, eggs, salad and cauliflower, they found no xanthins. By use of these latter foods they determined the endogenous urinary purins in a number of cases, and obtained results of individual constancy. When the endogenous urinary purin was estimated, and the amount of the food purin also known, they were able to explain the relation of the food purin to the urinary purin, and knowing the amount of the food purin and the total urinary purin, they were able to caclulate the quantity of endogenous urinary purin. They state that with beef and liver one-half, with thymus one-fourth, and with coffee one-third of their purin-N appears in the urine as uric acid and xanthin bases.

Digitized by Google

The xanthin bases have not received much attention as to the cause of their occurrence in the urine. They are present only in small amounts, and research has been mainly directed to the investigation of uric acid. The terms "bases" and "acid" are, however, in this connection somewhat misleading, as uric acid is a tri-oxy-purin, and xanthin and hypoxanthin are respectively di- and mono-oxypurin, hence their relation is closer than their names signify, and they should be estimated together.

The Metabolism of Meat Purins.

The publication, in 1900, of Burian and Schur's interesting researches, led to my investigation of the metabolic changes occurring after the ingestion of those substances, other than thymus, liver, veal, beef and ham, which my earlier estimations had shown to contain considerable amounts of purin bodies. At first sight, it might be supposed that no differences would be found, but as the flesh of certain animals (rabbits, etc.) contains methyl-xanthins, actual results appeared preferable to inference.

Subject of experiment: Walker Hall, 33 years old, weight, 70 kilos (11st. 5lb.). Urine free from albumin and sugar. The 24 hours' collection was taken from and to 8 a.m., and the urines were preserved for repeated estimations by the addition of a few drops of $CHCl_3$. During the experiment ten hours' laboratory work was performed, and a walk of four English miles taken each day.

To obtain the average amount of endogenous purin excretion, a fixed purin-free diet, consisting of eggs, bread, milk, cheese, butter, rice and sugar was selected. Neither tea, coffee nor beer was taken. Table XIII. gives the results, and indicates the variations in the calorific and nitrogenous values.

Digitized by Google

TABLE XIII.

Days.	Diet.	Calories.	Food. Nitrogen. Approximate.	Urinary Purin N.
1.	10 eggs, 360 gm. bread, 1000 cc. milk, 80 gm. cheese, 60 gm. butter - - - - - -	2631	22·38	0·1611
2.	10 eggs, 300 gm. bread, 1000 cc. milk, 80 gm. cheese, 60 gm. butter - - - - - -	2490	21·60	0·1608
3.	10 eggs, 300 gm. bread, 1000 cc milk, 80 gm. cheese, 60 gm. butter - - - - - -	,,	,,	0·1560
4.	8 eggs, 360 gm. bread, 1000 cc. milk, 80 gm. cheese, 60 gm. butter, 100 gm. rice, 50 gm. sugar - - - - - - - - - -	3053	20·50	0·1636
5.	8 eggs, 360 gm. bread, 1000 cc. milk, 80 gm. cheese, 60 gm. butter, 100 g.m. rice, 50 gm. sugar - - - - - - - - - -	,,	,,	0·1660
6.	8 eggs, 360 gm. bread, 1000 cc. milk, 40 gm. cheese, 60 gm. butter, 50 gm. sugar, 50 g.m. rice - - - - - - - - - -	2898	17·80	0·1563
7.	8 eggs, 360 gm. bread, 1000 cc. milk, 40 gm. cheese, 60 gm. butter, 50 g.m. sugar, 50 g.m. rice - - - - - - - - - -	,,	,,	0·1654
8.	8 eggs, 360 gm. bread, 1000 cc. milk, 40 gm. cheese, 60 gm. butter, 50 gm. sugar, 50 gm. rice - - - - - - - - - -	,,	,,	0·1645

It thus appears that upon an almost purin-free diet an average of 0·1623 gms. purin-N. per day was excreted, or 0·4869 gms. in terms of uric acid and xanthin bases, or 0·3645 gm. uric acid and 0·0950 xanthin bases approximately. Such amount is apparently uninfluenced by slight alterations in the quantities of nitrogen and the calorific values of the food.

In table xiv., the same fixed purin-free diet was taken, with the addition of certain meats whose amounts are separately noted. That the effect of the increased purin ingestion might be at once evident, the principal meal was taken at 7-45 a.m., when the larger portion of the meat was eaten. A light lunch preceded the next meal at 4 p.m., when the remaining purin-rich food was consumed. Bed 10 p.m. The meats were weighed raw, then steaked with fat sufficient for the purpose, and every particle of gravy, etc., preserved.

Digitized by Google

Table XIV.

1901.	Urine.	Total Nitrogen.	Total Purin Nitrogen.	Uric Acid Nitrogen.	Xanthin-bases Nitrogen.	P_2O_5.	Remarks.
Oct. 9	1260	21·8232	0·3769	—	—	—	
,, 10	995	16·2486	0·2739	—	—	—	Usual mixed food with tea,
,, 11	1215	18·4294	0·3472	—	—	—	coffee, &c.
,, 12	1010	17·5210	0·2208	—	—	—	
,, 13	1170	20·2132	0·1611	0·1454	0·0157	2·6208	Purin-free fixed diet.
,, 14	1125	20·4975	0·1608	0·1380	0·0228	2·4712	
,, 15	1255	23·5683	**0·2650**	**0·2190**	0·0460	3·7650	450 gm. chicken ; *uratic deposit.*
,, 16	1110	20·4680	0·1798	0·1435	0·0353	3·3858	——
,, 17	1085	19·4215	0·1560	0·1280	0·0280	3·3743	——
,, 18	1415	23·7294	**0·2489**	**0·1995**	0·0494	3·8771	530 gm. plaice ; *urates.*
,, 19	1140	19·6536	0·1755	0·1644	0·0111	2·9153	——
,, 20	1010	18·2966	0·1636	0·1250	0·0386	2·6260	——
,, 21	1860	22·4680	**0·2480**	**0·2108**	0·0372	5·9520	400 gm beef.
,, 22	1450	18·2990	0·1898	0·1565	0·0333	2·4460	——
,, 23	900	16·8940	0·1660	0·1270	0·0390	2·5475	——
,, 24	1150	17·2368	**0·1857**	**0·1391**	0·0466	2·8980	408 gm. beans.
,, 25	1260	17·4630	0·1563	0·1261	0·0302	2·7468	——
,, 26	1150	17·8542	0·1654	0·1220	0·0434	2·6532	——
,, 27	2080	21·8025	**0·2218**	**0·1761**	0·0457	3·0784	1700 cc. lager beer (5 bottles)
,, 28	1100	19·7281	—	0·1224	—	2·7546	—— *urates.*
,, 29	1030	17·8625	0·1645	0·1250	0·0395	2·6150	——
,, 30	1120	21·5621	**0·2475**	**0·2038**	0·0437	3·5430	Usual mixed food.

Except when mentioned, only purin-free fixed diet was taken.

Digitized by Google

The total urinary purin-N during two days after the ingestion of 450 gms. of chicken = 0·4448, the endogenous purin for two days = 0·1625 × 2 = 0·3250, hence 0·1198 was derived from the purin bodies contained in the chicken. As table iv. shows that these = 0·2200 N, 54·4 % ef the food purin appeared in the urine as exogenous purin. 537 gms. of plaice contain 0·1707 purin-N, and the total urinary purin was 0·4244. This minus 0·3250 endogenous purin leaves 0·1004, or 58·7 % of exogenous origin. Similarly, 400 gms. of beef contain 0·2400 purin-N, the total urinary purin was 0·4378—this less 0·3250 endogenous purin = 0·1138 or 47·4 % of the beef purin appeared in the urine as exogenous purin.

It thus appears probable, that allowing for the variations which occur in different animals, as well as in separate species, the system excretes in the urine (within 48 hours) about one-half of the fish, fowl or beef purins contained in the food. These figures confirm the results of Burian and Schur as regards the metabolism of beef, and indicate that the purins or alloxur bodies of fish and fowl flesh undergo similar chemical or vito-chemical changes during their passage through the human body.

The Metabolism of Vegetable Purins.

As vegetables contain a high percentage of water, or increase their holding during the processes of cooking, they would appear of less practical importance in respect to their "purin" contents than meat food. Camerer,[23] however, showed that a vegetable diet increased the urinary alloxuric nitrogen, and von Noorden[107] states that patients on a diet of beans, asparagus and sauerkraut excrete almost the same amount of uric acid as with a meat diet, and Douglas[36] found that with a diet rich in peas, beans and lentils, the output of uric acid was 0·702 gm., as compared with 0·675 gm. with ordinary food, and concluded that vegetables poor in proteid diminished, and those rich in proteid increased the uric acid excretion.

The estimations contained in table vii., show that vegetables known to be rich in proteid matter are also rich in nuclein, and

Digitized by Google

those poor in proteid are also poor in nuclein. Herein, therefore, lies the solution of the hitherto supposed anomaly. Naturally the increase in digestive activity necessitated by the less digestible food may account for a slight rise in the endogenous purin amount, but the remainder is probably due to the purin-N of these foods. To put this conclusion to a practical test, additional experiments were made to determine the exogenous purins after ingestion of haricot beans. The beans, which were in a thoroughly dry condition, were first weighed, allowed to soak in water for twelve hours, then boiled and re-weighed. All the three subjects of the experiment ate from the same lot of beans, and at the same time; otherwise, during the whole experiment, the food consisted of eggs, milk, cheese, bread, butter, sugar and rice. Tables xv., xvi., and xvii. give the results obtained:—

TABLE XV.

W.H., age 33 years, weight 70 kilos, health normal. Urine free from albumen and sugar.

1901.	Urine.	Table N.	Total purin N.	Uric acid. N.	P_2O_5.	Remarks.
Oct. 23...	900...	16·8940...	0·1660...	0·1270...	2·620...	—
„ 24...	1150...	17·2368...	0·1857...	0·1391...	2·898...	408 gm. dried beans.
„ 25...	1260...	17·4630...	0·1563...	0·1261...	2·746...	—

TABLE XVI.

N., aged 32 years, 49 kilos. Healthy. Urine normal.

1901.	Urine	Total purin Nitrogen.	Uric acid Nitrogen.	Remarks.
Oct. 22...	960...	0·1478...	0·1180...	Purin free diet.
„ 23...	760...	0·1504...	0·1290...	„
„ 24...	670...	0·1589...	0·1348...	„ +75—90 grs. haricot beans.
„ 25...	560...	0·1095...	0·0979...	„ free diet.
„ 26...	1310...	0·2315...	0·1985...	„ Usual mixed diet, with coffee.

Digitized by Google

TABLE XVII.

M., aged 7 years, 22 kilos. Healthy and urine normal.

1901.	Urine.	Total purin Nitrogen.	Uric acid Nitrogen.	Remarks.
Oct. 22	535	0·1224	0·1013	Purin free diet.
„ 23	560	0·1170	0·0927	„
„ 24	570	0·1355	0·1177	„ +120–150 grs. beans.
„ 25	650	0·1072	0·0900	„
„ 26	510	0·1570	0·1463	„ Usual mixed diet.

In each case there was a distinct increase in the urinary purin on the day when the beans were eaten. The fæces from W.H. yielded 0·0407 N as exogenous purin-N. 408 gms. of haricot beans contain 0·0920 purin-N., therefore 0·0513 N was absorbed. The increase of the urinary purin over the normal endogenous factor (0·1625 N) was 0·0232, and represented 45·5 % of the food purin-N. In the case of N, there was an increase of 0·0090 N above the average normal endogenous quantity of 0·1250 N, an absorbed amount of bean-purin 0·0162 N, and thus an exogenous urinary purin of 56·2 %. With M, there was an increase of 0·0180 N absorbed, giving an exogenous urinary purin of 55 %.

Thus these estimations demonstrate both the presence of purin bodies in the pulses, and their relations with the functional excretions. They furnish an explanation of observations which have heretofore appeared to be improbable, and table viii. should therefore be of use in regard to the dieting of patients whose exogenous nuclein metabolism is deficient. At the same time the experiments point to the necessity for exact estimations of the individual metabolic activities.

Haig [51] has recently called attention to the effect of asparagus upon his body-functions. Asparagus contains a considerable quantity of "asparagin," and he contends that this substance undergoes easy conversion into uric acid. At any rate, after the ingestion of asparagus, his uric acid excretion showed a considerable rise in quantity, and the symptoms he so readily obtains quickly appeared. He took salicylic acid to remove the

Digitized by Google

uric acid from the circulation, and increased his usual quantity of potatoes, with the object of getting the blood into a good condition of alkalinity to dissolve the uric acid it contained, and hold it in solution until excreted." These measures were successful, and his uric acid excretion returned to its normal level. If, however, the asparagin was the cause of the augmented uric acid excretion, it is not easy to understand how the addition of potatoes to the diet facilitated its removal. Asparagin is one of the principal extractives contained in potatoes, and hence the added quantity would increase the amount of uric acid. But the cause underlying Haig's observations is much nearer to hand. Asparagus contains purin bodies, and the urinary purin is proportionately increased.

The results obtained in the study M are of particular interest, as I have been unable to find any record of the "*endogenous*" purin of a child of this age. They show that the processes of cell growth necessitate the formation, and as will be seen later the partial excretion of a greater amount of nuclear substances than prevail in the adult economy, and as no evidence exists that ingested nuclein is built up into cell constituents, it is obvious that the increased excretion is to some extent a measure of the intense vitality which the younger tissues possess, and which enables the production of the highly-complex nuclein from the ingested proteid.[20]

The Metabolism of Beverage Purins.

It has been observed that in the countries where fermented drinks are commonly used, the diseases due to perverted metabolism are more frequent than in those in which "distilled spirits" are consumed. Hence in gout and allied conditions, it is customary to forbid beer and porter, and to allow small quantities of whisky and rum. The question as to the effect of alcohol on metabolic processes is, however, not yet fully decided. Hammond,[48] in 1856, found that alcohol diminished the excretion of uric acid and urea after normal, insufficient and excessive diets. Riess,[116] in 1881, gave adult men

Digitized by Google

3—5 gms. of absolute alcohol per kilo-body-weight each morning for thirteen days, and observed a diminution of 15—16 % urea and uric acid. Herman,[54] in 1888, obtained no increase in the uric acid excretion after the addition of Burgundy, Bordeaux and Hungarian wines to the usual diet. Herter and Smith,[55] in 1892, pointed out that whisky was without influence on the uric acid elimination. V. Jaksch,[107] in 1894, stated that when alcohol was given to children in acute and chronic illnesses, the uric acid and urea were diminished. Laquer,[80] in 1896, observed a diminution of the total alloxuric nitrogen after large quantities of alcohol. Lebers,[82] in 1897, gave 400—1,000 cc. of Malton sherry to several persons upon a fixed solid and liquid diet, and observed a decreased amount of total uric acid nitrogen. v. Noorden,[107] in 1896, reported the case of a patient who took large quantities of alcohol without any alteration in the uric acid excretion.

On the other hand, Herter and Smith found an increase in the uric acid excretion after champagne, and Rosenfeld[12] had a patient whose uric acid rose considerably after a single bottle of beer. Chittenden[25] observed in dogs fed upon milk crackers and dried beef, a decided increase in the uric acid (0·028 to 0·0472 gms.) after the administration of an average dose of 2·5 cc. of absolute alcohol per kilo of body weight, but an important decrease in the total nitrogen output. Donogany und Tibald,[34] in 1895, fed dogs upon a fixed diet, and found that the daily addition of 9—30 cc. of absolute alcohol caused increased uric acid excretion. Later researches upon nuclein metabolism in dogs have shown that their liver activity is very pronounced, and that they excrete the greater portion of their nucleins as urea or the intermediate body, allantoin. As alcohol is a well-known cell depressant, a possible explanation may be found in the diminished activities of liver and kidney functions, and the consequent lessened excretion of urea due to the deficiency in the uric acid decomposition, and its resultant increased excretion. In man at first alcohol will in the same way depress cell functions, and lead to a diminished production

Digitized by Google

of uric acid. But as alcohol promotes oxidation, the decreased production, if any, will be small. The decomposition of the uric acid, however, may not be normal, as alcohol in the human subject diminishes the solubility of urates, and thus hinders their transmission to the liver as well as lessens their chances of oxidative katabolism by the depressed zymogenic functions. Hence the elimination of both exogenous and endogenous purins will be delayed or incomplete and their deposition in the tissues favoured.

But if alcohol produces decreased uric acid elimination, how can the pronounced increase after beer and fermented drinks be explained, for the alcohol they contain should diminish the excretion of uric acid, and favour its deposit in crystalline form? The key to the problem may perhaps be found in the fact that each litre of beer contains 0·05 purin nitrogen or 0·12 xanthins, and thus furnishes the precursors of the increased urinary purins. With the view to practically demonstrate this supposition, on a day when no laboratory work was undertaken, I drank $1\frac{2}{3}$ litres of lager beer. Table xiv. shows that the urine for the day amounted to 2080 cc., and contained 18·2640 gm. total N, 0·2218 total purin-N, and 0·1761 uric acid N. In the 1700 cc. of beer there would be 0·090 purin N, and my endogenous purin quotient was 0·1625. As the diet was "purin-free," it is reasonable to attribute the increase of 0·0583 N to substances present in the beer. The experiment, however, was not entirely free from objections. The quantity of fluid was greater than the daily average of 1000 cc. milk, and Laquer[80] has shown that after $1\frac{1}{4}$—$1\frac{1}{2}$ litres of water the alloxur N is increased. Additionally, it is contrary to my usual custom to take alcoholic beverages, and so the effects would be more pronounced; thus, although the general depression of nervous and metabolic functions would diminish the purin excretion, the dilatation of the smaller blood-vessels and capillaries might assist the removal of an abnormal amount of undecomposed trioxy-purin. The experiment was therefore repeated in the following manner. To one litre of lager beer, 1 cc. of strong H_2SO_4

Digitized by Google

was added, in order to preserve the solubility and identity of the purins, the alcohol distilled off, the remainder evaporated to 500 cc. and then neutralised. The whole was drunk at 9-0 a.m. on the day of the experiment, and a purin-free diet observed. Table xviii. gives the results obtained.

TABLE XVIII.

	Quantity of urine.	Total purin Purin nitrogen.	
Nov. 23	1080 cc.	0·1680	Purin-N-free diet.
„ 24	1150 „	0·1590	„ „
„ 25	1250 „	0·1800	„ +1 litre lager beer.
„ 26	990 „	0·1640	„

As each litre of beer contains 0·05 purin N, this second experiment shows an increase of 0·1800 − 0·1625 (normal endogenous factor) = 0·0175 or 35 % of exogenous purin due to the beer purins. The results of the second experiment show that the first yielded, through adverse conditions, too high an amount. The low percentage obtained in the second experiment led to an enquiry as to the form in which the purin bodies exist in beer, and further analyses were made in which the beer was acidified, the proteids removed by lead acetate, and after removal of the lead by H_2SO_4, the free xanthins were determined by the usual process. One litre of lager beer contained 0·0327 free-purin-N. This amount would raise the 35 to 55 %, and leave the question of the metabolism of the bound purins of beers to a later investigation.

Wines do not appear to contain any purin bodies, and their harmful properties in gout must be due to some other constituent. Luff[91] points out that the ethereal constituents do not lessen the solubility of the sodium biurate or hasten the decomposition of the quadriurate, and considers that certain wines cause alterations in the liver metabolism, and an increased production of glycocin. Others have attributed their effects to their acidity, but the acid wines, clarets, etc., appear the least harmful.

Digitized by Google

The *methyl-purins*, tea, coffee and cocoa, increase the total urinary purin, but do not markedly affect the uric acid excretion. Burian and Schur[21] found that 35 % of caffeine ingested reappears in the urine. Boudzynski, Gottlieb,[15] and Rost[120] observed that after *caffeine*, one-fourth, and after *theobromin* one-third, appears as methyl-xanthin in the urine. Krüger und Schmidt,[79] in 1901, gave a patient 0·5—1·5 gm. of *pure* caffeine per day, and observed that:—

After	50	mg.	caffeine,	33·3	%	appeared	in	the urine.
„	100	„	„	28·0	%	„	„	„
„	200	„	„	19·3	%	„	„	„
„	400	„	theobromin,	47·0	%	„	„	„

Thus the metabolism of the methyl purins appears to vary with the quantity ingested. The manner in which the methyl group is liberated by the cell protoplasm is said to determine the amount of stimulus which the tissues receive from these substances. The xanthin group, as already remarked, is almost without any excitatory action, and its metabolic end products are constant. Perhaps, therefore, the variations in the excretion of unchanged methyl-purins is dependent upon the amount of the total reactive energy they invoke.

The Purins of the Fæces.

That the fæces contain nuclein bodies has been long known, but in metabolic experiments it has not been customary to examine them for other than the total nitrogen. Weintraud,[149] in 1895, first called attention to the existence of small quantities of xanthin bodies in fæces. Later in the same year[150] he stated that 0·100—0·130 gms., and in 1896,[152] that 0·100—0·500 mg. were present each day. Petrén[113] found 0·0357 and 0·0680 gms. per 20 gms. of dried material from milk and mixed diets respectively. Umber,[157] on a diet of 500 gms. meat, 300 gms. bread, 80 gms. butter, two eggs and coffee obtained 0·0965 alloxur N (0·240 gms. xanthin), and with 2000 cc. milk, and 100 gm. meat 0·1034 alloxur N (0·288 gms. xanthins)

Digitized by Google

Burian and Schur[22] found 0·0100 purin-I (0·025 xanthin) on a purin-free diet, and Parker[109] observed 30—38 mgms. xanthins on milk, 49—65 mgms. on mixed, 60—80 mgms. on meat and 71 mgms. on carbohydrate diets. If these figures are correct, there exists wide differences in the constituents of fæcal excretions. Probably, however, the methods employed will account for the variations, as Weintraud and Umber used the Krüger-Wulf method, which does not entirely precipitate all the purin bodies, yet carries down other nitrogenous substances. Petrén, after boiling in 1 % H_2SO_4, removed the acid and albuminous bodies by barium hydrate and ammonium chloride. With this method, it is difficult to wash the precipitate free from carbohydrate substances, and I found it necessary to decompose the silver-purin by SH_2, and again precipitate with $AgNO_3$. Parker determined the purin-quantities in the first precipitate by Salkowski's methods. More convenient is the process used by Burian and Schur, but several modifications are necessary. These are included in the description of the method I employed.

The fæces were dissolved in a 1 % solution of H_2SO_4, dried on a water bath, and reduced to a fine powder. 10—20 gms. were boiled for five hours in 1000 cc. of 1 % H_2SO_4. After neutralisation and filtration, the solution was warmed and excess of copper sulphate and sodium bisulphite added. The resultant white-brown precipitate was well washed with water at 60° C., suspended in boiling water, and then decomposed by colourless potassium sulphide. The filtrate, after removal of the copper sulphide, etc., was evaporated in acid solution to about 200 cc., the purins precipitated by ammoniacal silver nitrate, and after thorough washing with water at 60° C., the nitrogen estimated by Kjeldahl's method. The filtrate from the first precipitate was decomposed, treated with $AgNO_3$, and any resultant nitrogen added to the amounts previously obtained. The daily fæces were differentiated by charcoal.

The origin of the fæcal " purins " is not quite clear. When a purin-free diet is taken, there is still an appreciable quantity present in the fæces. No xanthin substances have been found

Digitized by Google

in the fluids obtained from biliary fistula, and under normal conditions there is probably no excretion of metabolic end-products through the intestinal walls. But when the food is rich in purins, particularly when they exist as undecomposed nucleins, it is quite possible that there may be an unabsorbed residue, whose amount depends upon individual activities of the digestive secretions. Indeed, Corlette[26] has shown that the nitrogen present in a loop of small intestine is chiefly in a higher nitrogenous form than proteid, and consists of nuclein which by its stronger resistance to digestive processes, tends to accumulate. But there is no evidence that the bowel purin contents vary according to general metabolism. Attempts have been made to show that upon a purin-free diet, no free xanthins occur in the fæces, but the albuminous bodies cannot have been sufficiently removed, as I have always found free xanthins present. There must be some source of these bodies, other than the cell-nuclean, said to be shed from the intestinal wall. In a purin estimation of a pig's stomach, I obtained 0·0229 % of total purin-N, and thus 100 grams of intestinal mucous membrane entirely ablated each day would furnish the necessary amount of fæcal purin nitrogen, but it is impossible that such occurs. It is more likely that the demulcent or irritative effects of certain foods determine the amount not only of cell destruction, but also of the quantities of the several digestive secretions. Possibly the pancreatic juice contains a considerable amount of free-purin bodies, as well as undecomposed guanin.

Considerable difficulty thus exists in obtaining a positive average fæcal purin excretion, even upon a purin-free diet, and it has been found that carbohydrate foods induce higher amounts than meats. If, however, approximate figures could be found for an individual upon a fixed purin-free diet, and if this diet was maintained during the ingestion of large quantities of meats, any distinct increase in the excretion might be reasonably attributed to the additional food taken. Table xix. gives the results of an experiment under these conditions.

Digitized by Google

TABLE XIX.

Fixed diet of eggs, bread, milk, butter, cheese, rice..	Purin nitrogen of fæces.
Purin free	0·0342.
„	0·0310.
„ +450 gms. chicken	0·0840.
„ +537 „ plaice	0·0464.
„ +400 „ beef	0·0617.
„ +408 „ beans	0·0717.

Thus when the 450 gms. of chicken were eaten, the increase of fæcal purins was 0·0510 N, and so the total purin-N of the chicken 0·2200 is reduced to 0·1700 (*vide* table iv.), and the percentage of urinary purin would be raised to 63·05 %. Similarly, the 537 gms. of plaice exogenous purin is decreased by 0·0245 N, and its percentage excretion changed to 68·5 %. 400 gms. of beef caused an excess of 0·0267 N, and an increase to 53·3 % exogenous urinary purin.

Weintraud has stated that the purin excretion by the fæces is so small as to be neglected, and hence its consideration is omitted from contemporary studies. The above results, however, show that if *large* quantities of purin-rich foods are taken, or the digestive powers are weak or overstrained, any quantitative experiments should demonstrate the exact absorption as well as the excretion of nuclein substances.

These results, together with those of the urinary purins confirm, to some extent, the statement of Burian und Schur that 50 % of the purin contained in the food reappears in the urine. But such amount can only be taken as a broad average, and is applicable only to healthy individuals upon perfectly assimilable diets. Allow such factors to be relatively or absolutely altered, and the necessity is apparent for the determination of personal factors, if our knowledge of nuclein metabolism is to be put to practical use. The whole experiment, nevertheless, shows that fish, fowl and meats all invoke similar reactions in the tissues, and that by the aid of a table containing

Digitized by Google

their "purin-percentage," their quantitative relations to uric acid and the xanthin bases can be determined.

Although it is difficult to obtain nitrogenous equilibrium with great variations of food, table xx. shows that but little disturbance occurred. The number of eggs eaten each day precluded precise estimations of the food nitrogen, and the figures are therefore taken from König's analyses.

TABLE XX.

	Food N.	Urine N.	Fæces N.
Oct. 13	22·3	20·2132	1·3078
„ 14	21·6	20·4975	1·3078
„ 15	25·4	23·5683	2·1536
„ 16	21·6	20·4680	1·3078
„ 17	21·6	19·4215	1·3078
„ 18	26·1	23·7294	2·4080
„ 19	20·5	19·6536	1·0776
„ 20	20·5	18·2966	1·0776
„ 21	26·3	22·4680	2·1168
„ 22	20·8	18·2990	1·0776
„ 23	17·8	16·8940	1·0776
„ 24	22·4	17·2368	3·8896

THE RATE OF ELIMINATION OF FOOD PURINS.

The rate of elaboration and excretion of the exogenous purins, or, in other words, the minimum time the metabolic organs require to transform and transfer the mono- and di-oxypurin of the food into the tri-oxypurin of the urine, is of practical as well as of critical interest.

The views of Hopkins and Hope[59] that the immediate increase of uric acid after a meal cannot be due to the nucleins of the food, as they are so slowly decomposed, and of Jerome,[66] that any such increase is directly due to the alloxur bodies contained in the food, are so directly opposed, that additional evidence is imperative. If it could be shown that a certain quantity of fed purin was recoverable from the urine within a definite period, and that the usual meats contained much "free"

Digitized by Google

and little "bound" purin, the position of the former workers would be less tenable. Should, moreover, the time occupied by the vital oxidation and katabolism of the food purin be "constant" for healthy adults, then a "time standard" might be established, and variations therefrom serve as indicators of perverted metabolism. A test powder of hypoxanthin might thus be employed in the same way as the test meal and methylene-blue solution are used in the diagnosis of alimentary and urinary diseases.

The analyses contained in table v. show that the purins of beef, veal, ham, chicken, etc., exist *principally* as free "purins," and that the glandular organs, liver, thymus, etc., also contain an *equally* large amount of unbound purin-bodies. The results of tables x. and xi. are also interesting, as they show that the whole of the expected hypoxanthin nitrogen was voided in *four* hours, and that of the uric acid in *eight* hours. Hence my experiments tend to confirm the observations of Jerome, and indicate the need for further investigation as to the rapidity of purin metabolism. The reason for the difference in elimination-rate between hypoxanthin and uric acid is not clear; perhaps the oxidases or oxidising processes act best upon the mono-oxypurin, or this body enters more rapidly into combination with albuminous substances than the tri-oxypurin, uric acid, and is earlier oxidised.

Additionally, the experiments of Lœwi and Burian und Schur show that the excretion of the bound exogenous nucleins is generally spread over two or more days, and Lœwi [89] has more recently stated that while a small proportion of these bodies is decomposed in the intestine, the major part is directly absorbed. The slowness of their excretion emphasises the greater metabolic efforts they invoke, and suggests their contraindication in all conditions of deficient metabolism.

The Exogenous Purin Remainder.

We have so far only considered that portion of the food-purin, which after oxidation to higher forms in the tissues or

Digitized by Google

liver, or in both, appears in the urine in quantities representing about half of the amount ingested. We must now therefore ask what is the fate of the other 50 %?

The answer to this question depends upon the particular theory of uric acid formation we decide to adopt. If uric acid is a terminal metabolic product, then this unexcreted balance will be retained within the system, and lead to interstitial or cellular deposits. This supposition has long been the contention of Haig,[50] who apparently considers that uric acid exists in certain foods, and passes therefrom directly into the urine; and that its rate of elimination, together with that of the uric acid of distinct body formation, is determined by the urinary flow, and that its excretion bears a definite relation to the quantity of urea voided. My own experiments show, however, that the volume of urine may vary considerably, and yet the total purin and uric acid excretions remain constant, and that although the urea bears a direct relation to the amount of proteid in the food, the uric acid is entirely independent of it. Thus the urea-uric acid quotient is clinically worthless. Under abnormal conditions retention may occur, but as Haig himself admits the inaccuracy of his estimations of uric acid precursors in meats, and employs unreliable methods for determinations of urinary uric acid, his conclusions can hardly be accepted. The constancy of the endogenous factor in table xiii., and the ready appearance of the urinary purins after ingestion point to a nuclein metabolism the reverse of sluggish, and permit the conclusion that with ordinary food and normal metabolism, uric acid is not retained.

Upon the theory which regards uric acid as an intermediate metabolic product, a masterly resume has just been published by Burian und Schur,[22] and it is thus unnecessary to cite the entire literature of the subject. These writers have also shown that when cats and dogs are fed upon uric acid and xanthins, no trace of uric acid can be found in the blood, and only $^1/_{20}$th reappears as urinary purin. Large quantities of allantoin have however been met with in their urines. In rabbits glycocoll is

Digitized by Google

said to take the place of allantoin, and $\frac{1}{6}$th only of the ingested purin is excreted as uric acid.

Small quantities of allantoin have also been observed in normal human urine, and in the renal secretion and ascitic fluid obtained from cases of liver cirrhosis.[103] Swain [145] considers that allantoin may be looked upon as an intermediary product between uric acid and urea, and that under ordinary circumstances about 50 % of the uric acid produced is almost completely oxidized to urea, but that when the metabolic organs are unable to fully decompose the purin radical, more allantoin and less urea appear in the urine. Hence the variation of end products in the nuclein metabolism of dog, cat and man depends rather upon the extent of circulatory and metabolic activity than upon definite peculiarities. We may, therefore, infer that uric acid is now more generally regarded as an intermediary metabolic product, and that its appearance in the urine is due to incomplete decomposition rather than to complete synthesis.

The seat of the endogenous uric acid formation has been assigned by numerous workers to the spleen, kidney and liver respectively, but Mendel and Jackson consider that in mammals no one organ alone performs such functions. Its destruction, according to recent work upon the subject, takes place mainly in the liver,[22] but Wiener,[155] working with "organ" extracts, showed that such prepared from liver, kidneys and muscles were able to decompose uric acid. If his methods were reliable and his experiments correspond to intra-vitam processes, the tissues generally may share in the uric acid destruction. But the rôle of the latter must be small compared with that of the liver, and to this conclusion the later experiments of Burian and Schur distinctly point. Probably, therefore, the exogenous purin-remainder is decomposed chiefly in the liver, perhaps partly in the tissues, and finally excreted as urea, or as bodies intermediate.

The endogenous purin may undergo similar metabolism, and only 50 % of the total quantity produced appear as such in the urine. Without any intention to discuss the origin of

Digitized by Google

the endogenous uric acid—a subject apart from the present enquiry—table xxi. may be appended as a contribution to our knowledge of its source, as it indicates a relation between the body weight and the endogenous purin excretion, and points to the probability that the endogenous purin is the expression of that amount of nuclear or protoplasmic activity necessary for the maintenance of cell functions.

TABLE XXI.

	Age.	Weight.	Endogenous Urinary purin.	
Infant	8 days	4·5 kilos	0·0170 Uric acid N.	Mares[96].
„	3 mths.	5· „	0·0330 „ „	Bendix[12].
„	12 „	8· „	0·0350 total purin N.	Camerer[24].
Female	7 yrs.	22· „	0·1200 „ „	Walker Hall.
„	32 „	48· „	0·1450 „ „	Walker Hall.
Male	23 „	68· „	0·1530 „ „	Burian[21].
„	32 „	70· „	0·1625 „ „	Walker Hall.
„	28 „	77· „	0·2020 „ „	Burian.

Digitized by Google

CHAPTER VIII.

The Pathology of Food Purins.

Although healthy blood does not apparently contain uric acid, its presence has been fully demonstrated in the following diseases:—

Pernicious Anæmia. Salomon, "Zeit. für Phys. Chemie.," S. 65, 1878.

Leukæmia. Klemperer, "Untersuchungen über Gicht.," S. 3, 1896.

Anæmia and intestinal inflammation. v. Jaksch, "Deut. Med. Woch.," S. 33, 1890.

Fevers. Malaria, between the attacks. „ S. 33, 1890.
Typhus, after febrile stage. „ „

Gout. Garrod, nature and treatment of gout, 1861.
M. Levy, "Verhand. f. Cong. f. d. Inn. Med.," S. 266, 1896.

Liver. Carcinoma, v. Jaksch.

Nephritis. Garrod, 100 cc. blood=4 mg. ur.
v. Jaksch.
Levy, "Virchow's Archiv.," S. 107, 1898, 12 cases.
Klemperer, in uræmic conditions.

Plumbism. Oliver, "Goulstonian lectures," 1891.

Pneumonia. v. Jaksch.

In cardiac, nephritic and pleural exudations it has also been found, and Bouchard[16] identified it in the nasal, pharyngeal, stomach, bronchial, vaginal and uterine mucus and the lachrymal secretions during uræmia.

Numerous estimations of urinary purins have been made in nearly all known pathological conditions, but only in the following have any distinct variations been demonstrated. Nephritis has yielded irregular results:—

Digitized by Google

Pathological Increases of Urinary Purins.

Uric Acid:

Alcoholism with enlarged liver. Strauss, "Zeit. für klin. Med.," p. 319, vol. 31.

Carbon monoxide poisoning. Münzer, "Deutsch. Archiv. f. klin. Med.," p. 236, vol. 52.

Cirrhoses of liver. v. Noorden, "Lehrbuch," p. 288. Also in a case of acute yellow atrophy.

Gout. M. Levy, "Berlin. klin. Woch.," p. 389, 1896, during and immediately after acute attacks.

Leucocythæmia. Ranke and many others.

Neurasthenia and Migraine. "His W., Verhand-Cong. f. inn. Med.," 1896.

Pneumonia. Herter and Smith, "New York Med. Journ.," 1892.

Sepsis. v. Jaksch, "Cbl. f. inn. Med.," p. 188, 1896.

Scurvy. v. Jaksch, "Cbl. f. inn. Med.," p. 188, 1896.

Xanthins:

Adipositas. Schreiber und Waldvogel, "Arch. f. Exp. Path. und Pharm.," 42, S. 74, 1895.

Diabetes. Baginsky und Sommerfeld, "Zeit. für Phys. Chemie., S. 412, 1895.

Diphtheria. Baginsky und Sommerfeld, "Zeit. für Phys. Chemie.," S. 412, 1895.

Scarlet fever. Baginsky und Sommerfeld, "Zeit. für Phys. Chemie.," S. 412, 1895.

Nephritis. Kolisch and Tandler, "Stuttgart," 1895.
Croftan, "New York Med. Journ.," Aug. 11, 1900.

Thyroidea. Schreiber und Waldvogel.

Pathological Decreases in Urinary Purins.

Uric Acid:

Anæmias. Brandenburg, "Berlin klin. Woch.," S. 137, 1896.
Honigman, "Cbl. f. inn. Med.," S. 873, 1897.
Taylor, " " " " "
Lœwy, "Cbl. f. inn. Med.," S. 188, 1896."

Gout. Decrease 1—3 days previous to attack. His (l.c.)
Vogt. S. (general decrease) "Deutsche Archiv. klin. Med., S. 21, 1901.

Digitized by Google

Dependent upon the extent of diuresis, variations occur in cardiac diseases,[115] and in epilepsy and chorea.[55] In diabetes, any increase is usually the result of excessive intake of meat purins. In certain fevers,[7] leucopenia,[63] phthisis,[107] and the intermediate stages of gout, the uric acid excretion is normal.[119]

These variations result from the balancing of production and destruction, or of production and excretion. Unfortunately, the great mass of statistics available for reference supply little distinct and decisive evidence; for until recently, diet was not considered in relation to the results obtained, and although the newer experiments give details of the food taken, there exist but few investigations in which the purin contents of the dietary have been estimated, and the reaction they incite, seriously recognised. It is even now generally thought that in gout the uric acid formation and excretion are normal, except before and after the acute attacks, and Watson[148] has demonstrated the fact by feeding a gouty patient upon thymus and nucleic acid, obtaining an increased uric acid elimination, and inferring that the nuclein metabolism was normal. The average daily uric acid was 0·307 gm. Thymus contains about 0·4 % purin-N, of which 0·1 % reappears in the urine as uric acid. On January 6th and 7th, 280 gms. of thymus were given, and the total uric acid excretion was 0·730, or 0·242 uric acid nitrogen. 280 gms. of thymus contain 1·12 gms. purin-N, of which 0·28 may be expected to appear in the urine as such. If we take the average excretion of 0·307, the total for the two days would be 0·614 uric acid, or 0·205 uric acid N, and the difference $0·242 - 0·205 = 0·037$ might be considered as due to the thymus. But 280 gms of thymus yield 0·28 exogenous urinary purin. Similarly, on January 9th, 252 gms. of thymus were administered, and the uric acid was still only slightly increased. There is no record of the total purin nitrogen or the xanthin bases, so that one cannot draw the conclusions desired, but if the many investigations upon the effect of thymus upon metabolism are correct, it is improbable that the patients' purin metabolism was normal, and possible that considerable retention occurred.

Digitized by Google

We can now, however, by aid of the average purin percentages of tables iv.—viii., and the knowledge of the metabolic results recorded in table xiv., deduct the exogenous portion from the total urinary purin, and so obtain the endogenous factor, or by the observations of a diet containing only purin-free foods, directly estimate the endogenous purin. As we may thus definitely assign some portion of the urinary purin to the presence of purins in food, we may, as the results of this investigation demonstrate, control the individual nuclein metabolism in its relation to purin ingestion by prescribing a dietary based upon quantitative purin estimations. Hence, so far as the reactive processes are concerned, the practitioner should now be able to precisely spare or stimulate his patient's metabolic powers, and under both conditions prognose the character and the intensity of the endogenous metabolism. To this end, indeed, further studies must now be directed. The experiments herein described have shown the importance of diet in its relation to uric acid, and the ease with which we may arrive at the amount of uric acid produced by the body itself, so that the future determinations of urinary purins should have for their object the attainment of definite factors for the endogenous purins, a knowledge of the circumstances that affect their variation, and the acquisition of data as to the rapidity and completeness of the individual exogenous purin metabolism. New estimations of the purin excretion in many diseases are by these conclusions rendered absolutely necessary, and the results therefrom should lead to more accurate conceptions of those metabolic changes, compensatory or otherwise, which probably accompany all pathological processes. The tendency to ascribe to uric acid a causative influence in the majority of the known diseases may be attributed to an over-enthusiasm, which has sadly enough induced much apathy upon any subject connected with uric acid, and consequently led many to under-estimate its rôle in the economy. When, however, pathological chemistry becomes gradually narrowed to the appreciation of cell-reactions, it may direct attention to the nuclear changes as the expression

Digitized by Google

of the dominating factor in cell life, and compel us to regard the endogenous purins as the more important indicator of metabolic processes, and the decomposition of proteids as dependent upon the energy liberated by the cleavage of cell-nucleins.

Still, at the moment, the source and function of the endogenous purin are not clearly delineated. A small portion may arise from leucocytic destruction, but distinct evidence of any other origin, although at present unavailable, will doubtless soon be furnished. Apart from theoretical considerations it is certain that diseases of perverted metabolism are frequently accompanied by abnormally increased or decreased excretion of urinary purin, and just as the secret of successful treatment in tuberculosis depends on its early recognition, so the radical therapeutics of metabolic diseases will depend upon our better knowledge of their earlier stages. Let the now fashionable precepts of individuality in treatment have further application in the practical recognition of a personal factor in the normal and compensatory chemical processes which occur in the human body, and the necessity for lifelong studies of patients and their families becomes at once apparent. Who is to obtain the information as to the metabolism of children of gouty parents and its alterations during their growth? The laboratory worker is unable to make material advances along this line, for the cases he is able to investigate are principally those of healthy, abstemious adults, with perhaps a sprinkling of kindly disposed patients. It is the clinician who alone can furnish the necessary information and statistics. If one may be permitted to further indicate the lines of such research, I would remark that opportunities to estimate a patient's endogenous purin, and occasions for systematic determinations of these factors from year to year in children of certain diatheses, must occur to many practitioners. The accumulation of such statistics would not only contribute to our general knowledge of these processes, but would often throw much light upon local conditions.

The occurrence of abnormal quantities of the xanthin bases

Digitized by Google

in the urines of nephritic and gouty patients has been pointed out by Kolisch and others, but their results were obtained by the (for this purpose) inappropriate Krüger-Wulff method, and as His,[57] and Schmoll have failed to find any such increases in gout, additional work is necessary before the theory of the causation of nephritis by alloxur bodies can be accepted. The opponents of the theory are not, however, justified in their denial of its probability. Already cited facts certainly show that in rabbits, unaccustomed to much purin in their foods, degenerative tissue changes occur, and normal growth is hindered. Whether these are due to direct toxic action, or to cellular exhaustion consequent upon overwork is a matter not yet decided. But Mitchell-Bruce[17] and Bier[13] are both agreed that metabolic perversion throws extra strain upon the vascular and excretory organs, and as a result of some chemical irritant, the heart hypertrophies and possibly the kidney degenerates. The relation, if any, between such excitant and the food purins, is a subject which I hope to further investigate.

Digitized by Google

CHAPTER IX.

The Estimation of Urinary Purins.

Although uric acid may be considered more as one of the normal excretions than an extensive factor in the causation of disease, there are many conditions in which a knowledge of a patient's nuclein metabolism is of practical as well as of theoretical value. Recent investigations have shown that a period of diminished uric acid excretion precedes acute attacks of gout, and if such alterations could be rapidly and easily observed, preventive measures might palliate if not avert the attack. The value of individual metabolic quotients has been already emphasised, and the possibility expressed that such information might lead to the earlier recognition of progressive changes in the chemical activities of the liver. This would at the same time indicate the possibility of any calculous deposits. Such evidence would also assist in the elucidation of the sources of endogenous purin, and of those conditions which excite or depress the synthesis and katabolism of nucleins.

The estimation of uric acid is not difficult, but it requires considerable care and attention, a certain amount of apparatus and much more time than the clinical assistant or general practitioner is able to bestow. The reliable methods which may be used are as follows:—

1. *Ludwig-Salkowski*—precipitation and removal of phosphates—precipitation of total purins as silver-magnesium salt—decomposition by H_2S or potassium sulphide—acidification and evaporation of the filtrate, crystallisation of the uric acid—drying-weighing, or estimation of the N by Kjeldahl's process.

2. *Hopkins.* Saturation of 100 cc. urine with 30 grms. NH_4Cl, after 1—2 hours, ppt., washed and heated to 90° C, acidified, evaporated to 30 cc. and the uric acid crystallised, dried and weighed.

Modifications:—

Ritter, G.[117] After solution of the ammonium urate, the addition of 20 cc. strong H_2SO_4, and titration whilst warm

Digitized by Google

against $^1/_{20}$ N $KMnO_4$. (1·578 $KMnO_4$ in 1 litre distilled H_2O) 1 cc. = ·00375 grm. uric acid.

Wörner E.[156] During precipitation the urine heated to 30—40° C, the ppt. washed with hot 10 % $(NH_4)_2$ SO_4 solution, the ammonium urate dissolved in 1—2 % NaOH solution, and heated on a waterbath until all NH_3 is driven off, and the N of the evaporated solution then estimated by Kjeldahl's method.

Lewandowsky, M.,[86] first ascertains by titration the acidity of the urine, and then makes it nearly neutral. In acid urines the formation of the ammonium urate is slow and imperfect.

Folin und Shaffer,[40] employ a solution containing 500 gm. $(NH_4)_2$ SO_4, 5gm. uranium acetate, 60 cc. 10 % acetic acid, 650 cc. distilled water. 75 cc. of this solution are added to 300 cc. of urine, 10 cc. NH_4OH added, and the whole allowed to stand until the next day. The ppt. is washed with 10 % $(NH_4)_2$ SO_4 solution, then dissolved in 100 cc. H_2O, 15 cc. H_2SO_4 added, and the 115 cc. titrated against $KMnO_4$.

Jolles, A.,[67] after formation of the ammonium urate, removes the ammonia by heating with magnesia for 45 minutes, then adds 10 cc. 1·4 % H_2SO_4, and oxidises the uric acid to urea by $KMnO_4$. To the urea solution NaOH and bromine are added, and the N evolved collected in an azotometer.

All these methods are, however, too laborious for clinical use. As we have previously seen, both uric acid and the xanthin bases are the result of an undestroyed or un-oxidised portion of the total amount of purin bodies dealt with by the system. If food substances are taken that contain methyl-purins, only the xanthin portion of the urinary purins is increased, and if they are absent from the dietary the quantity of xanthin bases in the urine is very small. If no methyl xanthins are present, the total purin-N represents more fully than the uric acid alone, the purin end products of nuclein metabolism. Hence, although the estimations of endogenous uric acid are valuable, much more so are those of the total urinary purins.

If, then, some method for estimating the total purins

Digitized by Google

were devised, which would occupy little time and yet give approximately accurate results, it would be of much practical use. The methods of Haycraft, Herman and Camerer for the estimation of the total alloxur bases offer no easier means to this end. The silver-magnesium precipitate is gelatinous in character, falls only slowly, and takes many days to become firm enough to occupy a constant space. In a long glass tube of $1\frac{1}{2}$ c.m. diameter, a magnesium-silver precipitate from 100 cc. urine

After 12 hours filled 27·5 c.m.
„ 36 „ „ 21·5 „
„ 64 „ „ 20·5 „

A small quantity of magnesium silicate was then added, and in 12 hours the precipitate had fallen to 18·4 c.m., and there remained constant. With a graduated measure, a similar precipitate from 50 cc. urine gave:—

After 24 hours 21 cc.
„ 48 „ 20 „

Magnesium silicate was then added, and in 10 minutes the precipitate had sunk to 15 cc.

The solution was next well shaken, and

15 minutes later the precipitate = 17 cc.
24 hours „ „ „ = 20 „

at which point it became constant. *Many* similar experiences yielded equal results. 0·01 uric acid was precipitated by Camerer's method and $\frac{1}{2}$ grm. talc added.

12 hours later the precipitate = 35 cc.
24 „ „ „ „ = 33 „
48 „ „ „ „ = 32 „
64 „ „ „ „ = $31\frac{1}{2}$ „
88 „ „ „ „ = 31 „
112 „ „ „ „ = $30\frac{3}{4}$ „
136 „ „ „ „ = $30\frac{1}{2}$ „
178 „ „ „ „ = 30 „
202 „ „ „ „ = $29\frac{1}{2}$ „

Digitized by Google

Oct. 18, 0·01 uric acid 24 hours after precipitation = 34 cc.
„ 20, 0·01 „ 24 „ „ = 34 „
„ 21, 0·01 „ 24 „ „ = 34½ „

It would thus appear that it is possible to obtain the silver-magnesium-purin precipitate as a firm, constant mass, as well as in gelatinous form. Should later experiments confirm this conclusion, it might be practicable to determine a specific value for each cc. of the precipitate, and so calculate from its amount the approximate quantity of purins present in the urine. But the reagent which serves as a medium to produce this result must itself occupy a constant space and be sufficiently fine and ponderous to overcome the varying densities of the solutions in which it acts. Talc appears to satisfy these two demands, and is without any reactive influence upon the other constituents. By the use of this medium I have obtained the following results:—

100 cc. of various urines Purin N estimated by Camerer's method. (Kjeldahl N).	100 cc. urine. Silver—magnesium—purin—talc precipitate.
0·0299	25 cc.
0·0287	24 „
0·0258	23 „
0·0153	13 „
0·0150	13 „
0·0162	15 „
0·0152	15 „
0·0174	15⅛ „
0·0162	15 „
0·0128	12 „
0·0130	12 „
0·0184	16 „
0·0168	14½ „
0·0124	12 „
0·0106	11 „
0·0221	21 „
0·0244	23 „
0·0163	15 „
0·0170	16 „
0·0259	24 „
0·0202	18½ „

From these figures it would appear that the precipitate maintains a somewhat constant relation to the amount of N found

Digitized by Google

by Kjeldahl's method, and gives a factor of 0·0011 N per cc. The following table states the results obtained by the use of this factor compared with those found by Camerer's method. Three separate estimations of 100 cc. urine were made at the same time. Compared with a similar estimation by Camerer's method, the constancy of the quantity of the silver-purin-talc precipitate confirms the previous figures.

Table XXII.

Amount of Precipitate.

Subjects of experiment.	Measure I.	Measure II.	Measure III.	Measure IV. Silver ppt. without talc.	Calculated N value.	Camerer N.
Dr. K. - - -	24 cc.	23 cc.	24 cc.	28 cc.	0·0258	0·0244
Prof. S. - - -	15 ,,	15 ,,	15 ,,	22 ,,	0·0165	0·0163
,, S. - - -	15 ,,	16 ,,	15 ,,	—	0·0170	0·0170
Väktmästare, J. -	24 ,,	25 ,,	24 ,,	31 ,,	0·0269	0·0259
Patients from Serafimer lazarettet.						
1. Pleurisy - -	19 ,,	18 ,,	19 ,,	31 ,,	0·0203	0·0202
2. Cardio-sclerosis -	10 ,,	11 ,,	10 ,,	—	0·0115	0·0116
3. Polyneuritis -	13 ,,	13 ,,	13 ,,	—	0·0143	0·0160
4. Acute nephritis -	4 ,,	5 ,,	4 ,,	—	0·0049	0·0030
5. Chronic alcoholism	10 ,,	10 ,,	11½ ,,	—	0·0121	—
6. Cardiac disease -	4 ,,	4½ ,,	4 ,,	—	0·0046	0·0033

For the opportunity to examine these pathological urines I am indebted to the Physicians of the Serafimer Lazarett, Stockholm.

Expressed as total quantities of daily purin excretion, the results of the two methods show close approximation:—

	Quantity of urine.	Measured ppt. N.	Camerer N.	In terms of uric acid and xanthin.
Dr. K.....	1000 cc.	... 0·2580	... 0·2440	... 0·7320
Prof. S.....	1250 ,,	... 0·2062	... 0·2047	... 0·6131
V. J.....	1200 ,,	... 0·3228	... 0·3108	... 0·9324
Patient				
1.....	1150 ,,	... 0·2340	... 0·2323	... 0·6969
2.....	1500 ,,	... 0·1725	... 0·1730	... 0·5190
3.....	2000 ,,	... 0·2860	... 0·3200	... 0·9600
4.....	1800 ,,	... 0·0680	... 0·0540	... 0·1720
5.....	2000 ,,	... 0·2420	... —	... 0·7260
6.....	1200 ,,	... 0·0430	... 0·0396	... 0·1290

Although I cannot assume that the factor 0·0011 N per cc. of purin precipitate is absolutely correct, yet it will form a

Digitized by Google

basis for the work of other observers, and lead, I hope, to the establishment of a definite factor. In the cases cited above, however, it gives very close results, and the figures agree most closely when the quantity of the urine approaches the normal. If the amount of urine and its specific gravity are markedly abnormal, the precipitate falls differently. The use of talc to a great extent overcomes this difficulty; concentrated urines may be easily diluted, and dilute urines may be evaporated to any desired quantity in slightly acid solution. As Camerer's process involves the use of ammonia to fully dissolve the silver-chloride formed, the question as to the varying amounts of sodium chloride in pathological urines is important, for in order to obtain a near uniformity of density, the amount of fluid must not exceed a certain number of cc. Additionally, any excess of ammonia interferes with the proper precipitation of the purin bodies. As to the silver chloride, I have in many instances added 20 drops of concentrated HCl to 60 cc. of urine, and experienced no difficulty with the solution of the chlorides in NH_4OH. In a case of chronic alcoholism, however, I was unable to entirely dissolve the silver chloride, and the part remaining fell with the purin precipitate, increasing its amount. A proposal to overcome this difficulty will be advanced later. Albuminous urines must be freed from albumin by boiling in slightly acidified solution. Medium amounts of sugar do not affect the precipitation, but with very large amounts, it is better to precipitate the urine with excess of copper sulphate and sodium bisulphite, decompose the precipitate by H_2S or K_2S, and then reprecipitate with ammoniacal silver nitrate solution.

The method I have used is as follows:—After noting the total daily quantity, the urine is tested for albumin. If present, this is removed by *slight* acidification with acetic acid and boiling. Two solutions are necessary:—

No. 1 solution.		No. 2 solution.	
Ludwig's Magnesium Mixture	100 cc.	Silver nitrate	1 grm.
Ammonia solution 20%	100 cc.	Ammonia (strong)	100 cc.
Talc	10 grm.	Talc	5 grm.
		Distilled water	100 cc.

Digitized by Google

To 90 cc. of the urine, 20 cc. of No. 1 solution are added. An immediate precipitate of the phosphates falls, and the clear fluid may be removed by filtration or decanted into another graduated measure glass. This precipitate should not be allowed to stand longer than 15—30 minutes, as otherwise the uric acid may be partially precipitated. To 80 cc. of the filtrate 18 cc. of solution No. 2 are added. The resultant precipitate is a mixture of silver chloride and silver purin; the former body is dissolved by the excess of ammonia. The filtrate should be shaken until all white flakes disappear and the finely granular yellow-white precipitate remains suspended. If the AgCl is not entirely dissolved, strong NH_4OH is added drop by drop and the solution well shaken until no AgCl remains. The measure-glass is then corked and placed in a cupboard or corner protected from strong light. After an hour the purin precipitate will have entirely fallen, but it is best to wait 24 hours before reading the result. If the precipitate occupies 7 cc., then this amount multiplied by 1·3 and 0·0011 = percentage quantity of purin N = 0·0100 %, and multiplied by the total daily amount of urine, say 1500 cc. = 0·1500 N or in terms of uric acid and xanthin bases = 0·45 gm. Should it now be desired to estimate the precise total purin N by Kjeldahl's method, this may be easily performed after the precipitate has been washed with water (60° C.) until the filtrate is neutral to lacmus paper, or the the exact amount of uric acid may be estimated in the precipitate by the Ludwig-Salkowski method. The process does not interfere with the details of the ordinary laboratory methods, but yields an approximate result which for clinical purposes is sufficiently accurate, and allows the estimation to be attained at little cost of time and apparatus. Still, two measure-glasses are necessary, and the trouble of preparing filter paper, and the process of filtration occupy a certain amount of time. It would be of advantage therefore, to have an apparatus in which the whole process could be completed easily. I think that the " purinometer " I

Digitized by Google

now propose will meet this requirement. It consists essentially of three parts:—

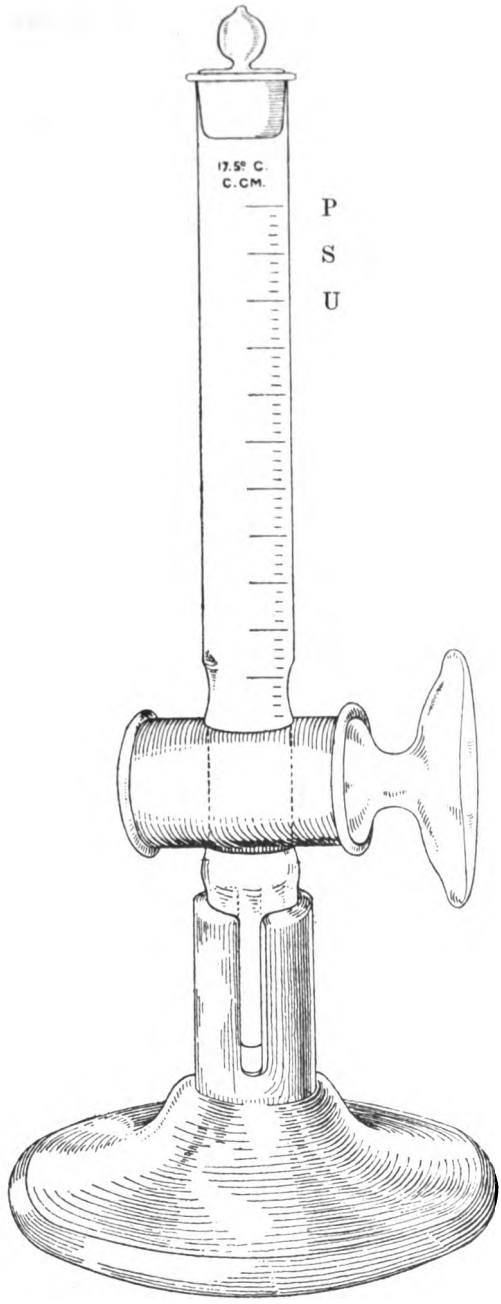
1. A closed, graduated tube.
2. A stop-cock, with a bore of the same diameter as the upper tube.
3. A small glass reservoir of known cubical capacity.

It is used in the following manner:—With the stop at a right angle to the tube, urine is poured in up to the mark *U*. The stop-cock is then turned parallel with the tube, and the lower chamber and the bore of the tap become filled with the urine. Solution 1 is then added to the mark *P*, and the precipitate allowed to settle. If the reagent contains no talc, the precipitated phosphates take a long time to settle, and there is some loss of uric acid. To demonstrate the time saved by the addition of talc, an experiment may be cited. At 3-30 p.m. (Nov. 14), 60 cc. urine, 7 cc. Ludwig's magnesia mixture and 7 cc. 20 % ammonia solution were placed in two graduated measures. To one of these 5 cc. of a 5 % talc solution was added.

P.m.	With talc.	Precipitate.	Without talc.
3·35	phosphate precipitate	= 18 cc...	phosphate precipitate = 72 cc.
3·40	„ „	= 16 „...	„ „ = 70 „
3·45	„ „	= 14 „...	„ „ = 30 „
3·50	„ „	= 13 „...	„ „ = 22 „
3·55	„ „	= 11½ „...	„ „ = 17 „

The precipitate of phosphates sinks into the lower chamber of the purinometer, and immediately this has happened the tap is again turned at right angles. To the clear fluid now remaining in the upper tube, solution No. 2 is added to the mark *S*. The resultant precipitate consists of a mixture of silver chloride and silver purin. The apparatus is then inclined backwards and forwards until the precipitate is yellowish-white. This can be readily seen by comparison with the white phosphate precipitate in the lower tube. The instrument is now allowed to stand 24 hours, when the percentage of

Digitized by Google



THE PURINOMETER.

Digitized by Google

purin may be read off at the upper level of the precipitate. Variations in the quantities of phosphates can also be observed by a similar grading of the lower chamber. An advantage may be claimed in the case of an excess of chlorides, which solution No. 2, or even a few added drops of strong ammonia will not dissolve. The silver chloride is heavy and falls rapidly (within a minute), whilst the lighter purin precipitate takes an hour or so to settle. It is possible, therefore, to allow the silver chloride to fall, turn the stop-cock, let the ppt. pass into the bore of the tap, and immediately re-turn the tap to a right angle. The loss of any purin-silver is by this means exceedingly slight, and the silver chloride excess does not interfere with the estimation. As the purinometer is graded also in cc., it can be used for ordinary measuring purposes when occasion demands. The temperature of the room in which the estimations are made should be between 10°—15° C.

The instrument is also of use for the collection and examination of urinary sediments for pus, casts, crystals, etc. When the insoluble substances have fallen, they can be passed into the lower chamber by slightly turning the stop-cock, and then, after emptying the upper chamber, be transferred to an object-glass, or if tubercle bacilli are suspected, directly centrifugalised in an ordinary tube. In both cases work is facilitated.

By use of the centrifuge, the precipitates become constant in a few minutes, and there is no necessity for the addition of talc. Where such apparatus exists, the convenience of the method is increased and the results are more regular. A graduated centrifugalising tube is however necessary.

Digitized by Google

CHAPTER X.

Summary of Results and Conclusions.

Of the known purin bodies, hypoxanthin, xanthin, adenin, guanin, and the methyl-xanthins, caffeine and theobromin are found in foodstuffs, and uric acid, and traces of xanthins and methyl-xanthins are met with in the urine.

1. As the methods available for the estimation of purin-bodies in animal organs were unsatisfactory, both as to technique and results, modifications were introduced and a reliable process worked out.

2. In foodstuffs, the purin-bodies occur in two forms, "*free*" and "*bound*." Both the glandular and muscular tissues contain approximately equal amounts of "*free*-purins," but the glandular tissues yield very large and the muscles only very small quantities of "*bound*-purins" (nucleins).

3. The estimations of the purin bodies contained in meats, show that considerable quantities are present, but that little difference exists between the amounts contained in white and dark meats.

4. Certain vegetable foods have been found to contain purin bodies. Amongst these are peas, beans, oatmeal, asparagus and onions. This furnishes a reason for the high uric acid excretion which follows their ingestion.

5. From several varieties of beer and porter purin-bodies have been isolated, and their percentage amounts estimated. Their presence may account for the harmful influence of these beverages in gout, and for some of the pathological changes which occur in chronic alcoholism.

6. Experiments upon the action of the purin bodies upon carbohydrate metabolism show that caffeine induces an in-

Digitized by Google

creased elimination of CO_2. Uric acid and hypoxanthin however, are inert in this regard.

7. The continued daily injection of hypoxanthin into rabbits hinders their growth, causes degenerative cell changes in the liver and kidneys, alters the cellular relations and contents of the blood and marrow, and produces slight changes in the intima of the smaller blood-vessels.

8. Feeding experiments with fish, fowl, beef, haricot beans and beer, under appropriate conditions, show that the urinary purin is increased in all these cases; that this increase corresponds with 50 to 60 per cent. of the purin bodies ingested with the food; that the purin is principally in the form of uric acid, and that the increase of urinary purin reflects the metabolic activity of the individual in regard to " nucleins."

9. The fæces may contain unabsorbed nucleins as well as certain purin substances from the digestive juices and cell-nuclei, and estimations of these bodies should be included in all metabolic experiments.

10. When the " free " purins are ingested, they are rapidly oxidised and decomposed. About 50—60 per cent. of hypoxanthin leaves the body as urinary purin (principally uric acid) within 4—6 hours, and the same percentage of uric acid appears in the urine after 8—10 hours. The " bound " purins, however, take 1—2 days before they are fully excreted.

11. The remaining 50 per cent. of the food-purin is excreted as urea, or as bodies intermediate between uric acid and urea.

12. By the quantitative estimations of purin-bodies in foodstuffs, an exact forecast of the exogenous urinary purin is possible, and its amount can be limited when necessary by prescribing a certain diet. From the total urinary purin the exogenous portion can be deducted and the endogenous amount obtained. Tables iv., vii. and viii. should, therefore not only be useful for dietetic purposes, but save a considerable amount of laboratory work.

Digitized by Google

13. The endogenous purin is partly derived from leucocytes, but mostly from the cell changes which result in the maintenance of bodily functions, as its amount is proportional to the weight of the individual; in other words, it bears a direct relation to the metabolic activity necessary for systemic purposes. Hence, as the cell-nucleus is the dominating factor in metabolism, the cleavage of cell-nucleins may incite the decomposition of proteid matter. It is possible that the endogenous urinary purin represents about one half of the total endogenous purin produced, and that the latter quantity indicates the extent of metabolic processes more completely than any other factors at present available.

14. The general conclusions of the investigation point to the need for determinations of the *endogenous* purins in many diseases, either by the use of purin-free food, or by the aid of tables giving the percentages of purins in foodstuffs, and the necessary calculations therefrom. In order to assist the clinician in his attainment of such records, an instrument—the purinometer—is proposed, and the method for its use is described.

15. The action of the purin bodies upon the alimentary system, as demonstrated by Pawlow and later by Potapow-Procaitis, strongly contra-indicates the employment of meat extracts or soups in hyper-chloridia.

Digitized by Google

REFERENCES.

For perusal of the following works I am indebted to the librarians of the Leipziger, Carolinska Institut's, Svenska Läkaresällskapet's, Svenska Vetenskaps Akademien's, British Medical Association's, Owens College, and Manchester Medical Society's Libraries. I express my thanks for their courtesy and help.

1. Ach, N. Uber die diuretische Wirkung einiger purinderivate. "Arch. f. Exp. Path. und Pharm." 44, 1900.
2. Albanese, O. Ueber das Verhalten des Caffeins und des Theobromins im Organismus. "Arch. f. Exp. Path. und Pharm." s. 449, 1895.
3. Allen and Searle. The bromine method. "" Analyst," p. 223, 1897.
4. Anten, H. Action diurétique de la Caffeine et de la Theobromine. "Arch. de Pharm. et de Therap." l. 465, 1901.
5. Archangelsky, W. Wirkung von Caffee und von Thee auf Atmung und Herz. "Arch. de Pharm. et de Therap." p. 425, 1900.
6. Arnstein, J. Ueber die Bestimmung der Xanthin basen im Harn. "Zeit. für Phys. Chemie," s. 417, 1898; and "Centrbl. f. d. Med. Wissenchaft," 15, 1898.
7. Baginsky und Sommerfeld. Zur Kenntniss der Ausscheidung von Alloxurkörpern bei Erkrankungen des Kindlichen Alters. "Zeit. f. Phys. Chemie." s. 412, 1895.
8. Baginsky, A. Ueber das Vorkommen von Xanthin und Hypoxanthin." Zeit. f. Phys. Chemie." s. 396, 1883.
9. Baldi, C. Azione della Xantina in rapporto piü specialmente con la excitabilité. "La Terapia Moderna," 12, 1891.
10. Bauman and Bömmer. Ueber die Fallung der Albumosen. "Zeit. f. Untersuch. d. Nahrungsmittel," s. 106, 1895.
11. Bethe, A. Den Schuppen von Alburnus lucidus. "Zeit. f. Phys. Chemie." s. 472, 1895.
12. Bendix, B. Beiträge zum Stoffwechsel des Sauglings. "Jahrbuch f. Kinderheilkunde," s. 23, 1896.

Digitized by Google

13. Bier, A. Ueber die Ursachen der Herz-hyperthropie. "Münch. Med. Woch." 16, 1900.
14. Binz, A. Die Wirkung des Destillats von Kaffee und Thee auf Atmung und Herz. "Centrbl. f. inn. Med." 47, 1900.
15. Bondzynski and Gottlieb. Ueber methyl-xanthin, ein Stoffwechsel product des Theobromin und Caffein. "Arch. f. Exp. Path. und Pharm." s. 132, 1895.
16. Bouchard, Ch. Excretion de l'acide urique chez les Uricemiques. "C. R. de la Soc. Biol." l. 454, 1896.
17. Bruce-Mitchell. Lettsomian Lectures, 1901.
18. Brandenburg. Ueber die diagnostische Bedeutung der Harnsaüre und Xanthin im Harn. "Berlin Klin. Woch." s. 137, 1896.
19. Bunge, G. Ueber die Physiologische Wirkung der Fleischbrühe. "Pflüger's Archiv." s. 235, 1879.
20. Burian und Schur. Ueber nucleinbildung im Säugethierorganismus. "Zeit. f. Phys. Chemie." s. 60, 1897.
21. Burian und Schur. Der Derstellung von Purinkörper in der menschlichen Stoffwechsel. "Archiv. f. d. ges. Phys." s. 309, 1900.
22. Burian und Schur. Der Derstellung von Purinkörper in der menschlichen Stoffwechsel. "Arch. f. d. ges. Phys." s. 239, 1901.
23. Camerer, W. Zur Lehre von der Harnsäure und Gicht. "Deut. Med. Woch.," p. 356, 1891.
24. Camerer, W. Harnsäure und Xanthinkörper im menschlichen Harn. "Zeit. f. Biol." s. 78, 1891; "Zeit. f. Biol." s. 218, 1897.
25. Chittenden, C. H. The influence of alcohol on proteid metabolism. "Journal of Phys." p. 220, 1891.
26. Corlette, A. Excretion in the small Intestine. "Journal of Phys." p. 351, 1900.
27. Croftan, S. So-called Uric-acid lesions. "New York Med. Journal," August 11, 1900.
28. Croftan, S. Rôle of alloxuric bases in Nephritis. "Journal of Amer. Med. Sci." p. 592, 1900.

Digitized by Google

29. Cushny and Van Naten. On the action of Caffeine on the Mammalian heart. "Archiv. f. Pharm." Dec., 1901; "Journal of Phys." p. 49, 1899.
30. Demant, A. Zur Kenntniss der Extractive-stoffe der Muskels. "Zeit. f. Phys. Chemie." s. 387, 1879.
31. Devoto, S. Eine neue Art der quantitative Eiweiss-bestimmung. "Zeit. f. Phys. Chemie." s. 465, 1891.
32. Dickinson, W. "Allbutt's System of Medicine," p. 371, 1900.
33. Duckworth, Dyce. "Allbutt's System of Medicine," p. 407, 1899.
34. Donogany and Tibald. Ueber den Einfluss des Alkohols im Organismus. "Ungar. Arch. f. Med." s. 189, 1895.
35. Dominici, M. Sur le plan de structure du système hématopoiétique des mammiféres. "Arch. de Med. Exp. et d'anat. Path." p. 473, 1901.
36. Douglas, C. Observations upon the excretion of uric acid. "Edin. Med. Journal," p. 32, 1900.
37. Dreschel and Balke. Zur Kenntniss von Xanthinkörper. "Dissert." 1889.
38. Fére, Ch. Note sur l'influence du café sur le travail. "C. R. de la Soc. Biol." p. 627, 1901.
39. Filehne, W. Ueber einige Wirkungen des Xanthins. "Arch. f. Anat. und Phys." s. 72, 1886.
40. Folin and Schaffer. Ueber die quantitative Bestimmung der Harnsäure im Harn. "Zeit. f. Phys. Chemie." s. 56, 1901.
41. Fürth, v. Ueber die Eiweiss-Körper des Muskel-plasmus. "Arch. f. Exp. Path. und Pharm." s. 231, 1895.
42. Garrod, A. Treatise on Gout, 1883, and "A new view of the formation of uric acid." Proc. Roy. Soc., 1893.
43. Gaucher, A. Pathogenesis der nephrité. These de Paris, 1885. "Revue de Med." l. 367, 1886.
44. Gautier, A. Les Toxines, l. 264, 1896.
45. Gottlieb und Magnus. Ueber die Beziehungen der Nieren circulation zur diuresis. "Arch. f. Exp. Path. und Pharm." s. 223, 1900.
46. Green, A. Vegetable Physiology, p. 274, 1900.
47. Halliburton, W. Chemical Physiology. 1894.

Digitized by Google

48. Hammond, C. The physiological effects of Alcohol. "Amer. Journal of Med. Sci." p. 305, 1856.
49. Hammarsten. "Physiologiska Chemie." 1898.
50. Haig, A. Uric acid as a factor in the causation of disease. 1900.
51. Haig, A. The body as an analytical laboratory. "Brit. Med. Journal," p. 1078, 1901.
52. Hedbom, K. Ueber, die Eniwirkung Verschiedener Stoffe auf das isolirte Säugethierherz. "Skand. Arch. f. Phys." s. 165, 1895.
53. Heerlein, W. Dass Caffein und das Kaffee destillat in ihrer Beziehung zum Stoffwechsel. "Pflüger's Archiv." p. 165, 1892.
54. Herman, H. Harnsäure-ausscheidung von Nahrungsmitteln mit Rücksicht auf die gicht. "Deut. Archiv. f. Klin. Med." s. 279, 1888.
55. Herter and Smith. The excretion of uric acid in health and disease. "New York Med. Journal," 1892.
56. Hess und Schmoll. Ueber die Beziehungen der Eiweiss substanzen der Nahrung zur Alloxurkörper ausscheidung. "Arch. f. Exp. Path. und Pharm." s. 24, 1896.
57. His, W. Untersuchungen an Gichtkranken. "Berlin Klin. Woch." s. 970, 1896.
58. His und Hagen. Kritische Untersuchungen ueber der Nachweis von purin basen. "Zeit. f. Phys. Chemie." s. 351, 1900.
59. Hopkins and Hope. On the relation of uric acid excretion to diet. "Journal of Phys." p. 271, 1898.
60. Hoppe-Seyler, F. Apparat zur Messung der respiratorischen Aufnahme im Menschen. "Zeit. f. Phys. Chemie." s. 579, 1894.
61. Horbaczewski. Beiträge zur Kenntniss der Bildung der Harnsäure und des Xanthin basen. "Sitzunsbericht der k. Acad. d. Wiss. Wien." 1891.
62. Hutchinson, R. Food and the principles of dietetics, p. 63, 1900.
63. Hutchinson and Macleod. Alloxuric excretion in a case of Leucopenia. "Amer. Journal of Exp. Med." p. 541, 1901.
64. von Jaksch. Ueber Uricacidæmia. "Deut. Med. Woch." s. 741, 1890.

Digitized by Google

65. Jerome, W. J. S. The formation of uric acid in man, and the influence of diet on its daily output. "Journal of Phys." p. 124, 1898.

66. Jerome, W. J. S. Uric acid after certain foods. "Journal of Phys." p. 98, 1899.

67. Jolles, A. Ueber eine neue methode zur quantitativen bestimmung der Harnsäure im Harn. "Zeit. f. Phys. Chemie." s. 223, 1901.

68. Katz, J. Die mineralischen Bestandtheile des Muskelfleisches. "Pflüger's Archiv." s. 63, bd. 85.

69. Kemmerich, G. Untersuchung ueber die physiologische Wirkung des Fleischextracts. "Pflügers Archiv." s. 49, 1869.

70. Klemperer, A. Ueber die Wirkung des Caffeins auf die Muskeln. "Untersuchungen ueber Gicht." 1896.

71. Kölisch und Tandler. Monograph. 1895.
Kölisch und Dostal. Das verhalten der Alloxurkörper im pathologischer Harn. "Wien. Klin. Woch." 1895.

72. Kobert, W. Ueber den Einfluss verschiedener pharmokologischer Agentier auf die Muskel substanz. "Arch. f. Exp. Path. und Pharm." 15, 1882.

73. Kossel, A. Ueber Xanthin und Hypoxanthin." Zeit. f. Phys. Chemie." s. 428, 1881.

74. Kossel, A. Beiträge zur Chemie. des Zellkerns. "Zeit. f. Phys. Chemie." s. 248, 1884.

75. Kossel, A. Zur Chemie. des Zellkerns. "Zeit. f. Phys. Chemie." s. 7, 1882.

76. Kossel, A. Ueber Guanin and Beiträge zur Chemie. des Zellkerns. "Zeit. f. Phys. Chemie." s. 404, 1883.

77. Krüger, M. Zur Kenntniss des Adenins und Hypoxanthin. "Zeit. f. Phys. Chemie." s. 444, 1894.

78. Krüger und Wulff. Ueber eine methode zur Bestimmung der Xanthinkörper im Harn. "Zeit. f. Phys. Chemie." 20, s. 176, 1895.

79. Krüger und Schmidt. Ueber das Verhalten von Theobromin im Organismus. "Ber, der. deut. Chem. Gesell." p. 2677, 3336, 32.

80. Laquer, S. Ausscheidungs-verhaltnisse der Alloxur-körper im Harn. "Verh. f. d. Kong. inn. Med." 1896.

81. Latham, W. P. Croonian Lectures. 1886.

82. Lebers, H. Zur physiologie und pathologie der Harnsäure ausscheidung beim Menschen. "Berlin Klin. Woch." 44, 1897.

Digitized by Google

83. Léblond, A. Etude de la Caféine. Paris, 1883.

84. Lehman und Wilhelm. Besitz das Caffein und die Caffein-freien Kaffee-surrogate eine Caffeeartige Wirkung? "Archiv. f. Hygiene." s. 310, 1898.

85. Lehmann, K. Ueber die Wirkung des Fleisch extracts. "Archiv. f. Hygiene." s. 249, 1885.

86. Lewandowsky, M. Ueber die Wörnersche Methode der Harnsäure-bestimmung. "Zeit. f. Klin. Med." s. 199, 1900.

87. Levy, M. Beiträge zum Stoffwechsel bei Gicht. "Berlin Klin. Woch." s. 387, 1896.

88. Leyden, V. Ernährungs-therapie und Diatetik., s. 364, 1897.

89. Lœwi, O. Untersuchung ueber den Nuclein Stoffwechsel. "Arch. f. Exp. Path. und Pharm." s. 159, 1901.

90. Lœwy, J. Der Eiweiss Stoffwechsel in einem Falle von Anæmia Splenica. "Cbl. f. inn. Med." s. 983, 1897.

91. Luff, Arthur P. Gout, its Pathology and Treatment. 1898.

92. Lusini, V. L'action biologique et toxique des Xanthins. "Archiv. Ital. de Biol." p. 212, 1900.

93. Lüthje, S. Der Einfluss der Blei auf die Harnsäure Ausscheidung." Zeit. f. Klin. Med." s. 266, 1896.

94. Mark-Schnorf, F. Zur Physiologie der Verdauung. "Pflüger's Archiv." s. 143, 1901.

95. Malfatti. Ueber die Krüger-Wulff's Reaction. "Wien. Klin. Woch." s. 597, 1900.

96. Mares. Sur l'origine de l'acide urique chez l'homme. "Archives Slaves de Biol." p. 207, 1888.

97. Maurel, E. Influence des variations des Azotés de L'alimentation sur L'excretion de L'acide-urique. "C. R. de la Soc. de Biol." p. 427, 1901.

98. Mayer, P. Ueber den Einfluss nuclein-haltiger Nahrung auf die Harnsäure-bildung. "Deut. Med. Woch." s. 186, 1896.

99. Meischer, O. Physiologisch-chemische unter-suchungen ueber die Lachsmilch. "Arch. f. Exp. Path." 36, s. 111, 1896.

100. Minkowski, O. Untersuchungen zur physiologie und pathologie der Harnsäure bei Säugethieren. "Arch. f. Exp. Path." s. 375, 1898.

101. Mitchell, C. Flesh Meats. 1900.

102. Milroy and Malcolm. The metabolism of nucleins. "Journal of Phys." p. 106, 1899.

103. Moscatelli, R. Ueber den Zucker und Allantoin im Harn. "Zeit. f. Phys. Chemie." p. 203, 1889.

Digitized by Google

104. Münzer, E. Der Stoffwechsel des Menschen bei acuter Phosphorvergiftung. "Deut. Archiv. f. Klin. Med." p. 236, 52.

105. Neubauer. "Zeit. f. Analy. Chem." s. 33, 1867.

106. Neumayer, S. "Verhand. f. d. Cong. inn. Med." s. 424, 1896.

107. Von Noorden. "Verhand. f. Cong. inn. Med." s. 424, 186.
Von Noorden. Lehrbuch, Stoffwechsel. 1894.

108. Oliver. Lead poisoning. Goulstonian Lectures. 1891.

108. Offer and Rosenqvist. "Ueber die weissen und dunklen Fleisches." Berlin Klin. Woch, 44, 45, 1899.

109. Parker, W. Xanthines in the Fæces. "Amer. Journal of Phys." s. 37, 1901.

110. Parisot, A. Etude de l'action de la caffeine sur les functions motrices. Paris, 1890.

111. Pawlow, M. Die Arbeit der Verdauungs-drusen. Wiesbaden, 1898.

112. Potapow-Procaitis, M. Influence de quelques alimento sur la quantité et la qualité de suc gastrique. "These." Lausanne, 1901.

113. Petrén, K. Ueber das Vorkommen der Xanthin basen in den Fæces. "Skand. Arch. f. Phys." s. 315, 1898.

114. Petrén, K. Nachtrag. "Skand. Arch. f. Phys." s. 412, 1899.

115. Petrén, K. Nachtrag. "Arch. f. Exp. Path." s. 269, 1900.

116. Riess, J. Ueber den Einfluss des Alkohol auf den Stoffwechsel. "Zeit. f. Klin. Med." s. 1, 1881.

117. Ritter, G. von. Ueber die Bestimmung der Harnsäure. "Zeit. f. Phys. Chemie." 21, s. 288, 1896.

118. Roberts, Sir W. Chemistry and Therapeutics of Uric Acid. 1892.

119. Rommel, O. Die Ausscheidung der Alloxurkörper bei Gicht. "Zeit. f. Klin. Med." s. 200, 1900.

120. Rost, J. Ueber die Ausscheidung des Caffein inn. Harn. "Arch. f. Exp. Path." s. 71, 1895.

121. Rosenfeld und Bornstein. "Verhand. f. Cong. inn. Med." s. 321,

122. Salomon, G. Ueber die Verbreitung und Enstehung von Hypoxanthin und Milchsäure im Thierischen Organisms. "Zeit. für Phys. Chemie." s. 65, 1878.

123. Salomon, G. Ueber die Verbreitung von Hypoxanthin in Thierischen Organism. "Zeit. für Phys. Chemie." 2, 5, 65, 1879.

124. Santesson, G. Wirkung des Caffeins auf das Herz des Kaninschen. "Skand. Archiv. f. Phys." 12, 1902.

125. Saundby, A. Lectures on Renal Diseases, p. 170, 1896.

Digitized by Google

126. Salomon und Krüger. Die Alloxurbasen des Harns und ihre Physiologischer Bedeutung. "Deut. Med. Woch." 25, s. 97, 1899.

127. Schafer, E. A. Handbook of Physiology. Vol. I., p. 719, 1898.

128. Schreiber, E. Ueber die Harnsaüre. Monograph. Stuttgart, 1899.

129. Schreiber und Waldvogel. Beitrage zur Kenntnis der Harnsäureausscheidung. "Arch. f. Exp. Path. und Pharm." s. 74, 1895.

Schreiber, E. "Deutsch. Med. Woch." s. 41, 1897.

130. Schmiedeberg, O. Vergleichende Untersuchungen ueber die pharmakologischen Wirkungen einiger Purin. "Berichte der Deutsche Chem., Gessellschaft." p. 2550, 1901.

131. Schindler. Beitrage zur Kenntnis des Adenins, Guanins und ihrer derivate. "Zeit. f. Phys. Chemie." s. 439, 1887.

132. Schultze und Bossard. Zur Kenntnis des Vorkommen von Allantoin, Asparagin, Hypoxanthin und Guanin in der Planzen. "Zeit. f. Phys. Chemie." 9, s. 420, 1885.

133. Schultze und Bossard. Ueber das Vorkommen von Vernin. "Zeit. f. Phys. Chemie." 10, s. 326, 1886.

134. Senator. Nothnagel's Handbuch, 19, p. 231, 1898.

135. Senator. Ueber die Unterscheidung des Weissen und des dunklen Fleisches. "Berlin Klin. Woch." No. 45, 1899.

136. Sivén, O. Zur Kenntnis der Harnsäurebildung in Menschlischen Organen unter physiologische Verhaltniss. "Skand. Arch. f. Phys." x., 1900.

137. Smith, E. Experiments upon the action of food upon the respiration. Proc. Roy. Soc., 1859. "Lancet," p. 215, 1859.

138. Sondén och Tigerstedt. "Skand. Arch. f. Phys." bd. vi., 1894.

139. Stadeler. "Liebig's Annalen," bd. 116, s. 105, 1860.

140. Steudel, H. Die constitution des Thymins. "Zeit. f. Phys. Chemie." s. 241, 1901.

141. Steudel, H. Das Verhalten einiger pyrimidin derivate im Organismus, s. 255, 1901.

142. Strecker. "Liebig's Annalen," s. 137, 1858.

143. Strauss. Ueber die Beeinflussung der Harnsäure und Alloxurbasen ausschiedung durch die Extractivestoffe des Fleischen. Berlin. Klin. Woch." s. 710, 1896, and "Zeit. f. Klin. Med." s. 319, 1896.

144. Sundvik, E. Xanthinstoffe aus Harnsäure. "Zeit. f. Phys. Chemie." s. 131, 1898.

Digitized by Google

145. Swain, R. E. The formation of Allantoin from uric acid in the animal body. "Amer. Journ. of Phys." p. 38, 1901.

146. Taylor, E. The influence of various diets upon the elimination of uric acid and the purin bases. "Amer. Journal of Medical Sciences," p. 141, 1899.

147. Taylor, A. E. Beiträge zur Verwerthung der Krüger-Wulffsches method zur Bestimmung der Alloxur-körper im Harn. "Cbl. f. inn. Med." s. 873, 1897.

148. Watson, Chalmers. Metabolism in Gout. "Edin. Med. Journal," p. 103, 1900.

149. Weintraud, S. Ueber die Ausscheidung von Harnsäure und Xanthinbasen durch die Fäces. "Centrlb. f. inn. Med." s. 18, 1895.

150. Weintraud, S. Beiträge zur Stoffwechsel der Gicht. "Charité Annalen," s. 275, 1895.

151. Weintraud, S. Ueber Harnsäure im Blut und ihre Bedeutung für die Enstehung der Gicht. "Wien. Klin. Rundschau," s. 8, 1896.

152. Weintraud, S. Enstehung der Harnsäure im Säugethier-organismus. "Verhand. f. d. Cong. f. inn. Med." s. 190, 1896.

153. West, S. Granular Kidney and Physiological Albuminuria., p. 154, 1900.

154. Wöhler und Frerichs. "Annalen der Chemie und Pharm." bd. 65, s. 340, 1848.

155. Wiener, H. Zersetzung und Bildung der Harnsäure im Thierkörper. "Arch. Exp. Path." 42, s. 374, 1899.

156. Wörner, E. Ein einfachen Verfahren zur Bestimmung der Harnsäure. "Zeit. f. Phys. Chemie." 27, s. 70, 1900.

157. Ulrici, H. Ueber pharmakologische Beeinflussung der Harnsäure-ausscheidung. "Arch. f. Exp. Path." s. 321, 1901.

158. Umber, A. Ueber den Einfluss nucleinhaltiger Nahrung auf die Harnsäurebildung. "Zeit. f. Klin. Med." s. 174, 1896.

159. Vinci, L. Azione della Caffeina sulla pressione sanguine." "Arch. di Farmacolog. et Therap." 365, 1891.

160. Yeo, B. Food in health and disease. 1897.

161. Zuelzer, G. Ueber die Alloxurkörper ausscheidung im Harn bei nephritis. "Berlin Klin. Woch." s. 72, 1896.

162. Zuntz, O. Die Abscheidung der peptischen Verdauungs-produkte mittelst Zinksulfat. "Zeit. f. Phys. Chemie." 27, s. 219, 1899.

Digitized by Google

BOOKS RECEIVED.

22 F 91

THE PURIN BODIES

OF

FOOD STUFFS,

their Estimation, Action, and Significance.

BY

I. WALKER HALL, M.D.,

Assistant Lecturer and Demonstrator in Pathology, the Owens College; formerly Senior Demonstrator in Physiology at the Owens College, Victoria University.

MANCHESTER:
SHERRATT & HUGHES, 27, St. Ann Street.
LONDON: H. K. LEWIS.
1902.

Digitized by Google

Digitized by Google

Digitized by Google

Digitized by Google

Digitized by Google

OCT 25 1905

APR 14 1911

Digitized by Google

COUNTWAY LIBRARY
HC 36ZH 4

Digitized by Google